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Further Information on Sustained Yields from Fluctuating Environments[1]

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ABSTRACT

The conclusions of Ricker (1958) are confirmed in series of 200 generations in which environment effects are simulated. The best constant rate of exploitation is very close to the maximum equilibrium rate when there is no environmental variation. A higher catch is obtained when an optimum portion of the stock is reserved for escapement and the remainder is harvested up to the level of the maximum equilibrium rate. Still higher catches are associated with reserving an optimum portion of the catch for escapement and harvesting all of the remainder. In general, these kinds of benefits are greatest at higher levels of environmental variability.

The observations of Ricker (1958) on maximum sustained yields from fluctuating environments were based on calculations of 28 or fewer "generations" for each of 6 types of populations. This present paper provides confirmation of those results, based on 200 generations. The 6 types of curves were the same as those of Ricker (1958).

Curves A, B, C, employ the relation

$$Ƶ = \mathrm{W}e^{a(1-\mathrm{W})}$$

where A is respectively 1, 2, and 2.678. Curve F conforms to Curve B in the ascending limb but where $Ƶ > 1$ it is put equal to 1. Curve G conforms to Curve A beyond $\mathrm{W} = 0.4$ but below $\mathrm{W} = 0.4$ it is determined by

$$Ƶ = \mathrm{W}e^{1.5\mathrm{W}}$$

which approximates the exponential form of the empirical curve of Ricker (1958). Curve H is based on the reciprocal relation

$$Ƶ = \frac{\mathrm{W}}{1 + \mathrm{A}(\mathrm{W} - 1)}$$

where $\mathrm{A} = 0.9$.

Table II gives the catch and escapement characteristics of these curves in their deterministic form.

For simulation of environmental variability random normal deviates as generated in an IBM 1620 subroutine were used. The series of 200 random normal deviates which was used for each run is given in Table I. Scaling of deviates to provide a specified range of extremes was accomplished as in Ricker

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TABLE I. Two hundred random normal deviates produced in IBM 1620 subroutine, and employed to simulate environmental variability. (Read the Table row-wise.)

-.36919000	-1.30248600	-1.61594200	-.62315800	-.29373400
.98673000	-.94336600	-.98162200	-.32163800	.38698600
-.72135000	-.08824600	1.30869800	-.60411800	-.15629400
-.69343000	.06287400	-.94498200	.32940200	-.12357400
-.12951000	.30999400	.05733800	1.27892200	.28514600
.77041000	-.54688600	.11565800	.04444200	-1.13013400
.80633000	-.70776600	1.02997800	1.42596200	1.43058600
-.22175000	1.62735400	-.39970200	.22348200	-1.23269400
-.51383000	1.25847400	-.37338200	1.23700200	-.31997400
.73009000	-1.01440600	-2.09106200	-.73347800	-.03125400
.31001000	-1.39128600	.24725800	-.88795800	.43346600
.02593000	.92783400	.44157800	-.42643800	-2.12581400
-1.32215000	2.74295400	-.70810200	1.45108200	.09090600
.06577000	.85407400	-.40178200	.54460200	-.11637400
-1.01031000	-.93880600	.16053800	.65412200	-.94765400
1.24961000	2.16431400	-1.22114200	.57964200	-.60293400
-.35447000	-.03656600	-.74682200	.12116200	-.28221400
1.97745000	1.25855400	.38349800	1.07868200	.81450600
.04537000	.84967400	-.03018200	.25220200	-.51277400
.64929000	-.46320600	-.18786200	-.55827800	-1.46405400
.58921000	.11991400	-.28954200	-.55275800	1.76066600
.66513000	-.60096600	1.46477800	.06876200	-.03861400
-.32295000	-.82584600	-1.12490200	.10628200	2.93810600
.42497000	-.75472600	-.25858200	1.35980200	-.50917400
-1.29111000	-1.58760600	.86373800	1.62932200	-.58045400
.32881000	-.52448600	.04205800	.71484200	-.47573400
.08473000	1.23463400	.07637800	1.41636200	.60498600
.77665000	.48975400	-.23330200	.53388200	.46170600
.20457000	.04087400	1.91301800	-.13259800	-1.10557400
-.83151000	-.31200600	-.68466200	-.78307800	.70314600
.46841000	-.76888600	-.22634200	.38244200	-.31213400
-1.09567000	-.52976600	1.08797800	-2.83603800	1.64858600
.27625000	.20535400	.05829800	-.63851800	-.61469400
-.61583000	-.76352600	.48461800	.77500200	-1.30197400
.02809000	1.36359400	-1.83306200	2.20452200	-.61325400
2.00801000	-.61328600	-.09474200	2.45004200	.25146600
.12393000	.10583400	.49957800	.31156200	-.90781400
1.17585000	-.67904600	.74989800	.58908200	-.29109400
-.03623000	-1.16792600	-.54378200	-.91739800	-.09837400
-1.71231000	-.56080600	-.58146200	.59212200	.47034600

(1958) by multiplying the deviates by an appropriate factor. The absolute quantity was then augmented by one and the deviates used as multipliers or divisors depending on sign. Level A of variability uses random normal deviates, for which 19 out of 20 lie in the range of effects between $\frac{1}{3}$ and 3 times the mean. Level B provides a comparable range of effects from $\frac{1}{5}$ to 5 times the mean.

Effect of Level A of Variability Upon Yield

Table III confirms the observation that when stocks are harvested at the appropriate rate for maximum equilibrium catch, imposing environmental variability does not influence the mean catch from Curves A, B and C, slightly

Table II. Maximum equilibrium catch, rate of exploitation, and size of spawning stock, under conditions of no environmental variability for 6 populations (from Ricker, 1958).

Population	Maximum equilibrium catch	Rate of exploitation at m.e.c.	Size of spawning stock at m.e.c.
		%	
A	0.330	43.3	0.433
B	0.935	72.2	0.361
C	1.656	84.1	0.314
F	0.760	78.3	0.210
G	0.330	43.3	0.433
H	0.520	68.4	0.240

Table III. Catch and escapement statistics for 6 populations when subjected to random variability of Level A and when rate of exploitation is that appropriate for maximum equilibrium catch. Figures in parentheses are from Ricker (1958).

	Catch			Escapement		
Population	Mean	Standard deviation	Range	Mean	Standard deviation	Range
A	0.337 (0.33)	0.238	0.04–1.25 (0.09–0.90)	0.441	0.311	0.06–1.63
B	0.940 (0.94)	0.599	0.19–3.04 (0.31–2.13)	0.362	0.231	0.07–1.17
C	1.662 (1.66)	1.067	0.318–5.81 (0.62–3.67)	0.314	0.202	0.06–1.10
F	0.728 (0.71)	0.483	0.12–2.48 (0.22–1.65)	0.202	0.134	0.03–0.69
G	"Extinction" (<.0001) in 10 generations					
H	0.594 (0.58)	0.369	0.13–1.81 (0.20–1.25)	0.275	0.171	0.06–0.88

TABLE IV. Catch and escapement statistics with Level A of environmental variability, where available stock up to the optimum size of spawning population is reserved for escapement, and the fishery can take the surplus but not to exceed the rate of exploitation for maximum equilibrium catch for the stock as a whole. Figures in parentheses are from Ricker (1958).

	Catch				Escapement		
Population	Mean	Standard deviation	Range	Years of no catch %	Mean	Standard deviation	Range
A	0.372 (0.36)	0.309	0–1.370 (0–0.88)	16 (21)	0.613	0.292	0.224–1.794
B	1.032 (1.01)	0.756	0–3.773 (0.21–2.33)	0.5 (0)	0.494	0.215	0.322–1.453
C	1.826 (1.79)	1.282	0.116–6.605 (0.47–4.12)	0 (0)	0.424	0.183	0.314–1.249
F	0.901 (0.86)	0.612	0.051–3.084 (0.22–1.89)	0 (0)	0.296	0.133	0.210–0.855
G	0.35 (0.36)	0.309	0–1.370 (0–0.87)	19 (21)	0.592	0.294	0.152–1.794
H	0.628 (0.61)	0.441	0–2.110 (0.10–1.30)	0.5 (0)	0.345	0.156	0.237–0.975

TABLE V. Catch and escapement statistics with Level A of environmental variability, when available stock up to the optimum size of spawning population is reserved for escapement, and the fishery takes all the remainder. Figures in parentheses are from Ricker (1958).

	Catch				Escapement		
Population	Mean	Standard deviation	Range	Years of no catch %	Mean	Standard deviation	Range
A	0.471 (0.45)	0.524	0–2.573 (0–1.44)	19 (33)	0.416	0.045	0.199–0.433
B	1.189 (1.15)	0.960	0–4.742 (0.20–2.87)	0.5 (0)	0.360	0.002	0.338–0.361
C	2.042 (1.99)	1.463	0.200–7.450 (0.55–4.61)	0 (0)	0.314	. . .	. . .
F	0.987 (0.92)	0.741	0.051–3.728 (0.21–2.21)	0 (0)	0.210	. . .	. . .
G	0.438 (0.44)	0.517	0–2.573 (0–1.45)	22.5 (33)	0.408	0.059	0.152–0.433
H	0.670 (0.65)	0.561	0–2.751 (0.09–1.66)	0.5 (0)	0.240	0.003	0.198–0.240

decreases that for Curve F, slightly increases that for Curve H, and results in rapid extinction for Curve G. The range in catches, as might be expected, is wider in the series of 200 generations than in the series of 24 given in Ricker (1958). The agreement between the 2 series is otherwise extremely close, and the escapements are accordingly appropriate.

Statistics for partial stabilization of escapement, by harvesting surplus over escapement provided the rate does not exceed that for maximum equilibrium catch are summarized in Table IV. The agreement is again very close, the improvements in annual catch by comparison with those of Ricker (1958) given in parentheses, as follows: Curve A 10.4% (9%); Curve B 9.8% (7%); Curve C 9.9% (8%); Curve F 23.8% (21%); Curve H 5.7% (5%). Curve G is "rescued" from extinction and produces slightly more than what is obtained when there is no environmental variability.

With stabilization of escapement to the optimum (Table V), the benefits to catch are confirmed: Curve A 39.8% (36%); Curve B 26.5% (22%); Curve C 22.9% (20%); Curve F 35.6% (30%) and Curve H 12.8% (12%). The effect on Curve G is to increase catch by 30% over that obtained when there is no environmental variability. The greater variability in catch associated with these benefits is apparent, although the years of no catch for Curves A and G are not as frequent as in Ricker's shorter series.

EFFECT OF LEVEL B OF VARIABILITY UPON YIELD

Harvesting at the rate appropriate for maximum equilibrium catch, greater environmental variability does not influence average catch for Curves A, B and C, slightly decreases that for Curve F and, as in the case of a lower level of variability, increases the catch for Curve H (Table VI).

TABLE VI. Catch and escapement statistics with Level B of environmental variability, when rate of exploitation is fixed at that appropriate for maximum equilibrium catch. Figures in parentheses are from Ricker (1958).

	Catch			Escapement		
Population	Mean	Standard deviation	Range	Mean	Standard deviation	Range
A	0.338	0.382	0.006–2.146	0.443	0.501	0.008–2.810
	(0.33)		(0.03–1.07)			
B	0.936	0.944	0.058–4.846	0.360	0.363	0.022–1.866
	(0.93)		(0.14–3.12)			
C	1.654	1.681	0.106–7.732	0.313	0.317	0.020–1.462
	(1.64)		(0.27–5.72)			
F	0.734	0.783	0.172–4.172	0.203	0.217	0.005–1.156
	(0.73)		(0.08–2.35)			
G	"Extinction" (<.0001) in 7 generations					
H	0.695	0.656	0.055–3.394	0.321	0.303	0.025–1.568
	(0.68)		(0.10–1.96)			

The benefits from stabilization of escapement are much greater with the more variable environment. With partial stabilization (Table VII) (which was not included in Ricker (1958) for level B of variability except for Curve G), the mean catch compared to that of Table VI is increased for Curve A 23.7%, Curve B 25.9%, Curve C 26.8%, Curve F 51.9%, Curve H 14.5%. Extinction for Curve G is again avoided.

TABLE VII. Catch and escapement statistics with Level B of environmental variability, when available stock up to the optimum size of spawning population is reserved for escapement, and the fishery can take the surplus but not to exceed the rate of exploitation for maximum equilibrium catch for the stock as a whole. Figures in parentheses are from Ricker (1958).

	Catch				Escapement		
Population	Mean	Standard deviation	Range	Years of no catch %	Mean	Standard deviation	Range
A	0.418	0.495	0–2.273	33	0.683	0.551	0.094–2.976
B	1.178	1.299	0–6.689	13	0.587	0.407	0.081–2.576
C	2.098	2.253	0–11.561	4.5	0.508	0.351	0.053–2.186
F	1.115	1.122	0–5.384	1.5	0.375	0.262	0.150–1.492
G	0.209 (0.33)	0.398	0–2.241 (0–1.30)	64 (42)	0.367	0.492	0.001–2.934
H	0.796	0.807	0–3.773	7.5	0.444	0.314	0.145–1.743

TABLE VIII. Catch and escapement statistics with Level B of environmental variability, when the available stock up to the optimum size of spawning population is reserved for escapement, and the fishery takes all the remainder. Figures in parentheses are from Ricker (1958).

	Catch				Escapement		
Population	Mean	Standard deviation	Range	Years of no catch %	Mean	Standard deviation	Range
A	0.639 (0.59)	0.904	0–4.816 (0–2.15)	39 (42)	0.367	0.010	0.091–0.433
B	1.575 (1.51)	1.765	0–8.549 (0–4.80)	6 (4)	0.357	0.019	0.194–0.361
C	2.647 (2.55)	2.734	0–13.242 (0.24–7.57)	0.5 (0)	0.314	0.001	0.295–0.314
F	1.323 (1.20)	1.412	0–6.801 (0.06–3.67)	1.5 (0)	0.210	0.004	0.153–0.210
G	0.300 (0.44)	0.662	0–3.635 (0–2.15)	68 (42)	0.208	0.186	0.001–0.433
H	0.894 (0.86)	1.025	0–4.982 (0–2.80)	10 (8)	0.235	0.018	0.114–0.240

Complete stabilization of escapement produces even more substantial benefits (Table VIII), the increases, with those of Ricker (1958) in parentheses, being: Curve A 89.1% (79%); Curve B 68.3% (62%); Curve C 60.0% (55%); Curve F 80.3% (64%); Curve H 28.6% (26%). For Curve G there is an increase in catch from partial to complete stabilization of escapement. However, a gain over the condition of no environment variability (catch of 0.330) was not achieved, whereas in Ricker's series a gain was indicated. This is attributable to the difference in the two series of random numbers, which is reflected in the greater percentage of years with no catch. There were 3 long "runs" of no catch in the present series, one of 39 "generations", another of 35 and a third of 23.

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Laboratory Observations on Embryonic Development and Larvae of the Pacific Cod (*Gadus macrocephalus* Tilesius)[1]

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ABSTRACT

The relationship between rate of development of eastern Pacific cod eggs and water temperature was found to be linear at temperatures of 5–11°C. Time to 50% hatching ranged from 8.5 days at 11°C to 17 days at 5°C. Most successful hatching occurred at the lowest temperature. Newly hatched larvae from 5° water had an average length of 4.45 mm.

INTRODUCTION

Pacific cod (*Gadus macrocephalus* Tilesius) inhabiting waters off the Canadian coast are close to the southern limit of their range in the northeastern Pacific. They exhibit features of growth and mortality which appear to reflect the stress of a high-temperature environment (Ketchen, 1961). As part of the continuing program to determine factors which limit the abundance of cod in Canadian waters, experiments have been conducted on the effects of temperature on the embryonic development and hatching of eggs.

PROCEDURE

On March 13, 1962, eggs were obtained from ripe cod taken by trawl net on a spawning ground at Nanoose Bay in the Strait of Georgia, British Columbia (Lat. 48°15′N, Long. 124°W). Fertilization was effected by stripping eggs and milt into a plastic container of sea water and gently stirring the mixture. The fertilized eggs were transported by ship to the laboratory and within 1 hr of fertilization had been transferred to 4 wide-mouth Erlenmeyer flasks (500 ml) containing sea water with salinity approximately 26.5‰. The flasks in turn were placed in 4 constant temperature baths (5, 7, 9 and 11° C) maintained within ±0.1° C by thermoregulators. Water in the flasks remained unchanged during the course of the experiment, but aerators were used in each. Small numbers of eggs (10–15) were sampled periodically from each flask to record development and, as larvae hatched, they were removed and placed in other containers.

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RESULTS

ADHESIVE PROPERTIES

Yamamoto and Nishioka (1952), and Mukhacheva and Zviagina (1960), have demonstrated that the eggs of the cod in the northwestern Pacific area are demersal (sink to the bottom) and slightly adhesive. Thomson (1963) has confirmed that the eggs of eastern Pacific cod are also demersal.

When the fertilized eggs from Nanoose Bay (average diameter 1.02 mm, range 0.98–1.08 mm) were placed in the flasks they sank and adhered to the bottom of the flasks. However, unlike eggs of Pacific herring (*Clupea pallasii*) and lingcod (*Ophiodon elongatus*), cod eggs do not retain their adhesiveness for the entire period of embryonic development. In these experiments (1962) time of loss of adhesiveness was not noted other than the fact it had been lost by the time hatched larvae were being removed. In experiments conducted in 1963 the adhesive characteristics were lost at about 30 hr after fertilization. Tamura (1952), Mukhacheva and Zviagina (1960), and others, have recorded an increase in diameter of cod eggs after fertilization. It is possible, therefore, that the adhesiveness is a temporary phenomenon caused by imbibition.

RATE OF DEVELOPMENT

Development time, in days, was noted according to certain arbitrary, easily identified stages. These stages were:

I. Migration of germ ring past equator (Fig. 1c);
II. One-half circumference of yolk surrounded by embryo (Fig. 1d);
III. Three-quarter circumference of yolk surrounded by embryo (Fig. 1e);
IV. Embryo full circle (Fig. 1f);
V. Larva first hatched (Fig. 1i). (Time of first hatching was not actually observed but it was considered to be half way between the time of the observation when larvae were first observed and the time of the immediately preceding observation.);
VI. Larvae 50% hatched. (This was defined as the time when one-half of all those which subsequently did hatch, *had* hatched.)

TABLE I. Time of development (days) to certain stages for Pacific cod eggs held at various temperatures.

Stage of development (see text)	Temperature (°C)			
	5°	7°	9°	11°
I	2.9	...	2.0	...
II	5.0	3.3	3.0	<3.0
III	6.5	5.0	4.0	3.0
IV	7.9	6.9	5.0	4.0
V	14.9	12.0	8.9	7.9
VI	16.9	12.8	10.5	8.6

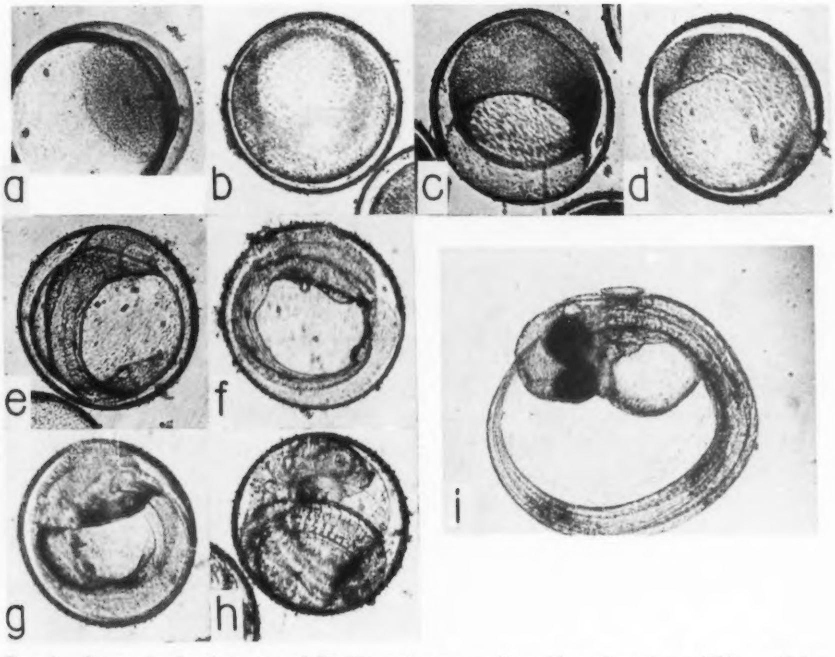

FIG. 1. Stages in development of Pacific cod eggs: a. Late blastodermal cap (7°C — 45 hr); b. Early germ ring (5°C — 70 hr); c. Migration of germ ring past equator (5°C — 70 hr); d. Embryo approximately ½ circle (7°C — 70 hr); e. Embryo ¾ circle; somites and optic lobes readily distinguishable, tail growing free of yolk sac (11°C — 70 hr); f. Embryo full circle (5°C — 189 hr); g. Tail of embryo starting past head (7°C — 167 hr, eye pigmented); h. Embryo with tail well past head (7°C — 189 hr, heartbeat evident); i. Newly-hatched larva (7°C — 333 hr).

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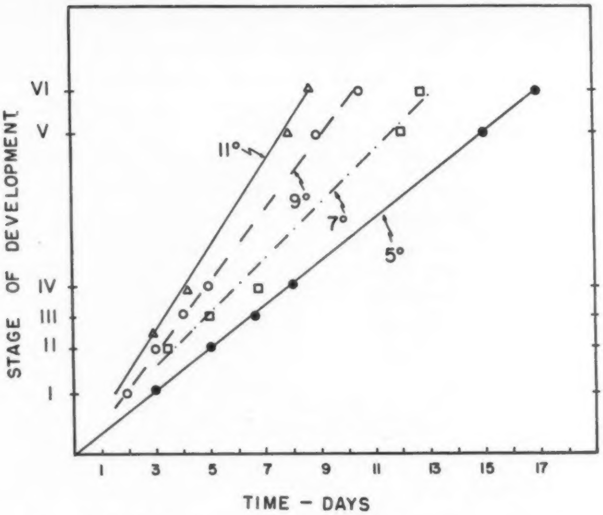

FIG. 2. Time of development (days) to certain stages for Pacific cod eggs held at various temperatures.

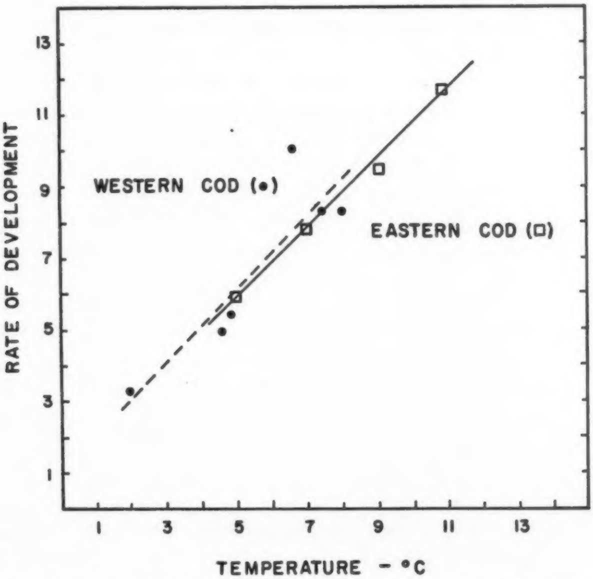

FIG. 3. Rate of development to hatching time for cod eggs from the eastern Pacific (Nanaimo, British Columbia) and western Pacific (data from Mukhacheva and Zviagina, 1960). The ordinate scale is 100 times the reciprocal of the number of days to 50% hatching.

Development time for eggs held at the various temperatures is shown in Table I and plotted in Fig. 2. The data for development at 5°C have been arbitrarily plotted as a straight line in the figure, and data for other temperatures have been plotted against these same ordinates. Approximately straight lines were obtained for these other temperatures.

Development to hatching occurred in all tanks. The time to stage VI (50% hatching) ranged from approximately 8.5 days at 11°C to 17 days at 5°C. A straight-line relationship between water temperature and rate of development in various marine fish embryos was demonstrated by Krogh (1914). Using Krogh's notation of V as the velocity or rate of development (expressed as 100 times the reciprocal of *t*, the time in days from fertilization to 50% hatching), and T as the temperature in degrees Celsius, the data for 50% hatching time in Table I have been used in calculating a "velocity curve" which is shown in Fig. 3. The relationship is linear or close to linear and has the formula:

$$V = 1.113 + 0.954\ T.$$

Also shown in Fig. 3 are rates for various temperatures calculated for western Pacific cod from hatching data summarized by Mukhacheva and Zviagina (1960). The line calculated for western cod is close to that for eastern cod ($V = 1.052 + 1.013\ T$); the two obviously do not differ significantly.

SUCCESS OF HATCHING

To obtain a measure of the relative success of hatching at the various temperatures, all observed larvae were removed and transferred to other containers. Because the eggs were all on the bottom of the container, observation of the success of hatching was contingent upon the larvae emerging from the eggs and becoming visible; i.e. rising above the mass of eggs on the bottom. The larvae were seen to swim actively upwards after hatching and except where larvae were very numerous (5° and 7° flasks) removal with a pipette was accomplished with little disturbance to eggs. Table II shows the number of larvae removed at various intervals from each flask. Dead eggs remaining were counted at the conclusion of hatching. These, together with sampled eggs and larvae gave a total count of eggs originally placed in each flask. If we presume that eggs removed by sampling would have hatched in the same proportion as those not removed, then a measure of the success of hatching is obtained from larvae removed and dead eggs remaining.

Hatching was most rapid in 11° water (first observed at 189 hr) but was least successful. Less than 2% of the 2124 eggs hatched at this temperature. As water temperature decreased, per cent survival increased to 6.4 at 9°, 19.8 at 7° and 22.8 at 5°.

There were numerous abnormalities among embryos reared in 9° and 11° water, particularly in the latter. Abnormalities included such features as 2 tails or 2 heads and, more commonly, distorted development about the yolk sac (Fig. 4)[2].

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[2] Cod larvae with 2 heads were the most common of abnormal shapes observed by Yamamoto and Nishioka (1952, p. 302).

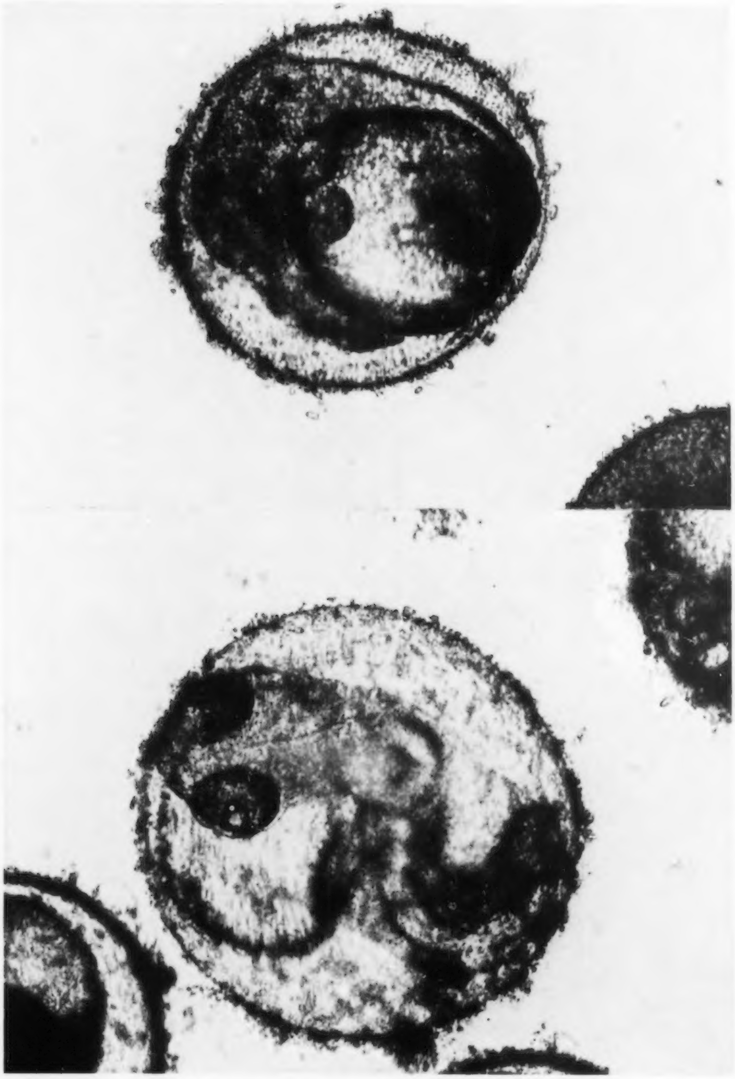

FIG. 4. Types of abnormal Pacific cod embryo. Note presence of ciliates and plant growth on surface of eggs (11°C — 189 hr).

TABLE II. Distribution of hatching at various temperatures as denoted by numbers of larvae removed.

Hours from fertilization	Temperature (°C)			
	11°	9°	7°	5°
167	0	...	...	...
189	2	...	...	...
214	21	2	...	...
239	10	23	...	...
259	1	43	...	...
294	...	39	50	...
308	...	...	155	...
312	...	...	41	...
333	...	...	118	...
357	...	...	10	6
361	...	...	3	1
379	...	...	...	58
389	...	...	...	54
406	...	...	...	172
433	...	...	...	155
456	...	...	...	117
475	...	...	...	11
479	...	...	...	3
Total number hatched:	34	107	377	577
Dead eggs remaining:	2000	1557	1528	1953
Percentage hatched:	1.7	6.4	19.8	22.8
Approximate number of eggs sampled:	90	105	150	210
Total number of eggs:	2124	1769	2055	2740
Approximate duration of hatching period (*hours*):	71	75	82	132

Ciliates and algae grew rapidly in the 11° water, less rapidly at 9° and slowly at 7° and 5°. Whether they affected egg development at any temperature is unknown. The removal of dead eggs, without disturbance to live eggs, was virtually impossible and the presence of dead eggs may have had an injurious effect on those still living.

A semi-logarithmic plot of percentage hatched against temperature is approximately inversely linear for 7, 9 and 11°C (Fig. 5). The curve to 5° suggests that a maximum survival is being approached. However, Yamamoto and Nishioka (1952) obtained 60% survival in western Pacific cod eggs at 3–6° (mean 4°) and 24% at 2°, and it is instructive, therefore, to consider possible reasons for the "low" percentage of hatching at 5°. Table II shows that, in actual numbers hatched, larvae were 1.5 times more numerous in the 5° flask than in the 7° flask. Removal of larvae from these flasks, because of the numbers involved and the fact that eggs were no longer adhesive, did result in some disturbance of eggs. If the number removed is assumed to be a criterion of the disturbance factor, then there was greater disturbance to hatching eggs at 5° than at 7°. Examination of dead eggs showed substantial numbers which

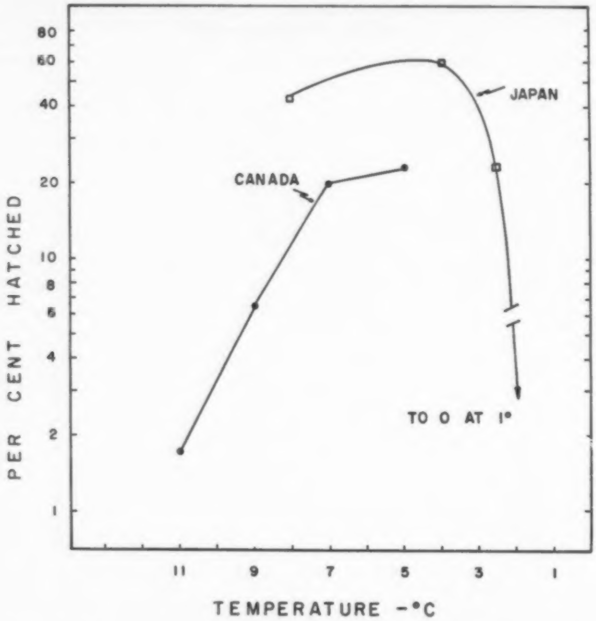

FIG. 5. Percentage of Pacific cod eggs hatched at various temperatures of development in experiments conducted at Nanaimo, British Columbia, and in experiments conducted by Yamamoto and Nishioka (1952) in northern Honshu, Japan.

appeared ready to hatch at 5° and 7° temperatures. Bonnet (1939) showed a period of high susceptibility to mortality just prior to hatching in Atlantic cod eggs and the same may be true for Pacific cod. Yamamoto and Nishioka (1952) have implied that this is so.

Another contributing factor to "low" survival at 5° may have been overcrowding of eggs in the flask. The 5° flask contained 2740 eggs which was more than in any other flask and about one-third more than in the 7° flask. In view of these possibilities, the percentage hatches shown in Fig. 5 are not necessarily associated with temperature as such; it is quite possible that under optimum conditions hatching would approach 100% at all the temperatures used.

DURATION OF HATCHING PERIOD AND DISTRIBUTION OF HATCHING

While observations of eggs and removal of larvae were not as frequent as desired, some indication of the duration of hatching at each temperature was obtained. The time of first hatching was considered to be the mid-point between the time of the observation at which larvae were first sighted and the time of the previous observation. Similarly, the time of last hatching was considered to be the mid-point between the time of the observation when last larvae were removed

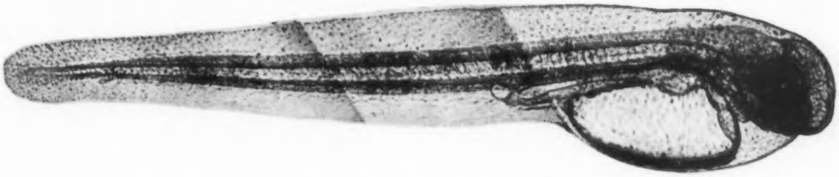

FIG. 6. Newly-hatched Pacific cod larva from 5° water at 357 hr; length 4.07 mm.

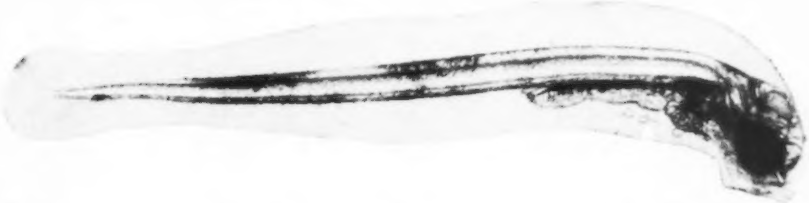

FIG. 7. Characteristic distribution of pigmentation in Pacific cod larva about 2–3 days after hatching.

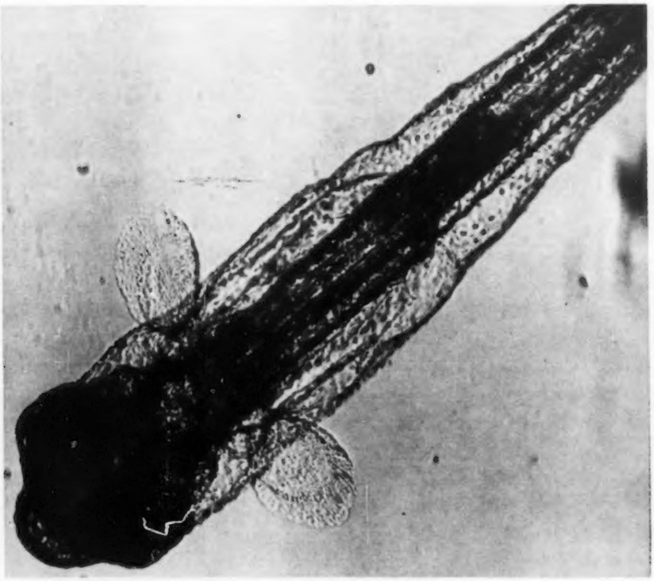

FIG. 8. Ventral view of anterior portion of newly-hatched cod larva showing position and size of pectoral fins.

a
f
h
v
8
r
l
[illegible]
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and the previous observation. For example, in Table II larvae in 7° water were first observed at 294 hr. As no larvae were observed at 259 hr, time of first hatching was estimated to be (259 + 294)/2 or 277 hr. Time of last hatching was (361 + 357)/2 or 359 hr. Thus, duration of hatching period is 359–277 or 82 hr at 7°. The durations of hatching at 11, 9 and 7° were 71, 75 and 82 hr, respectively. At 5°, however, hatching extended over a period of 132 hr. Reasons for the difference are not known. Distribution of hatching about the 50% hatching time was closely symmetrical in all but the 5° flask (Table III). At this temperature the distribution of hatching appeared to have a positive skew. A cumulative plot of number of larvae hatched against time for 5° showed an elapsed period of 14 hr between the time 25% of the larvae had hatched and the time when 50% had hatched. The time required to reach 75% from the 50% point was 26 hr.

TABLE III. Duration of the interval between hatch of 25% and 75% of the eggs which subsequently did hatch; also, column 3 shows the time from fertilization to 50% hatch.

Temperature	25–50% hatch	50% hatch	50–75% hatch	25–75% hatch
°C	*hours*	*hours*	*hours*	*hours*
11	11	206	10	21
9	15	252	13	28
7	7	306	8	15
5	14	405	26	40

Confirmation of differences in the duration of hatching, of the magnitude noted above, and tests for skewness in distribution of hatching would require more rigid control of the number of eggs and examination of eggs at constant time intervals. Observations at temperatures lower than 5° would also be desirable.

EARLY LARVAL STAGE

Newly-hatched larvae from 5° water had an average length of 4.45 mm (range 4.07–4.84 mm), larger than that recorded by Mukhacheva and Zviagina (1960) (average: 3.60 mm; range: 3.27–3.80 mm). On the basis of available information it is not possible to say whether the difference is due to temperature differences during incubation or to inherent biological differences. Nevertheless, description of the larvae conforms with that given by Mukhacheva and Zviagina (1960). The head is inclined ventrally, the mouth closed initially and the anus is open to the side of the larva posterior to the yolk sac (Fig. 6). Pigmentation is faint in newly-hatched cod but within 2 or 3 days becomes pronounced and is characteristically concentrated along the upper part of the body cavity and in two conspicuous bands on the tail (Fig. 7). The pectoral fins are approximately the size of the eye (Fig. 8). About 10 days after hatching the yolk sac becomes absorbed. Seven days after hatching the larvae had grown to a length of 5.24 mm, but measurements in this period were too few to establish a reliable growth rate.

Attempts to feed cod larvae with phytoplankton (***Skeletonema costatum*** and *Monochrysis lutheri*) and larval ***Artemia*** were unsuccessful. The only experiments in which Pacific cod larvae have been carried to the point of feeding were those of Yamamoto and Nishioka (1952) who reared larvae to a length of 6.8 mm (20 days after hatching) and found that preferred food was small crustacea and larvae of bivalve molluscs.

SUMMARY

1. Fertilized eggs of eastern Pacific cod (average diameter of 1.02 mm) were found to be demersal and slightly adhesive. Adhesiveness did not remain during the entire course of development to hatching.
2. The relation between rate of development (100 times the reciprocal of *t*, the time to 50% hatching) and temperature was found to be linear at temperatures of 5–11°C.
3. Time to 50% hatching ranged from 8.5 days at 11°C to 17 days at 5°C.
4. Under conditions noted hatching was least successful at 11° and attained a maximum at 5°C.
5. Duration of hatching time was 71, 75 and 82 hr at water temperatures of 11, 9 and 7°C. At 5°, hatching time lasted for a period of 132 hr.
6. Newly-hatched larvae had an average length of 4.45 mm (range 4.07–4.84 mm).
7. Absorption of the yolk sac at 5° occurred in approximately 10 days.

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The assistance of Messrs D. F. Alderdice and F. P. J. Velsen in the planning and maintenance of the experimental apparatus is gratefully acknowledged. Dr K. S. Ketchen, under whose direction the study was initiated, and Dr W. E. Ricker, were most helpful during preparation of the manuscript.

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Lethal Concentrations of Copper and Zinc for Young Atlantic Salmon[1]

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ABSTRACT

The toxicity of copper and zinc sulphates to immature Atlantic salmon (*Salmo salar* L.) was tested in soft water.

The relation between concentration of metal and survival-time could be fitted by a straight line when logarithms were used. A sharp break in this relation marked the *incipient lethal level*, where survival became indefinitely long. Incipient lethal levels were 48 μg/l of copper and 600 μg/l of zinc.

In solutions containing both copper and zinc, fish died twice as fast as would occur if the 2 metals were simply additive in their lethal action. Resistance-times in zinc solutions were increased at pH 7.9–9.3, and results fitted the hypothesis that dissolved zinc was toxic, but not suspended zinc. Survival in a given concentration of zinc was 4 times as long at 5° as at 15°C, and the incipient lethal level was at least 1.5 times higher, with fish acclimated to each temperature.

INTRODUCTION

Copper and zinc seem to be common pollutants in fresh water, and there have been many investigations of their toxicity to fish. Results have been summarized by Doudoroff and Katz (1953) and are heterogeneous. However, penetrating studies have recently been done by Lloyd (1960, 1961a, 1961b) and Lloyd and Herbert (1962) using rainbow trout. Among other things they showed with completeness how toxicity changes with water hardness. Most notably, Lloyd (1961b) was able to add up the toxic effects of copper and zinc in mixtures by expressing each concentration as a proportion of the "lethal threshold" instead of using conventional chemical units.

In a problem of copper-zinc pollution in northern New Brunswick, the major fish species affected was the Atlantic salmon. As a foundation for other studies on biological effects, lethal concentrations of the metals were determined for young salmon.

MATERIALS AND METHODS

Chemical procedures used in this work were similar to those in "Standard Methods" (APHA, 1960), and minor differences have been recorded in a manuscript (Sprague and Carson, MS, 1963). Hydrogen-ion concentration was measured with glass-electrode pH meters accurate to within 0.1 unit. Total hardness and hardness due to calcium were measured by titration with EDTA, and interference by metals was prevented by 2 ml of 2.5% KCN. Zinc was

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determined by the mixed-colour dithizone method, and copper by the sodium diethyldithiocarbamate method (APHA, 1955) with a carbon tetrachloride extraction (Sandell, 1959). Metal determinations were done 3 times on each sample, using a spectrophotometer. Tests showed that each metal was determined with satisfactory accuracy even in the presence of much higher concentrations of the other.

The water used in this work was taken from the supply of the town of St. Andrews, N.B., which comes from a soft-water lake. The only treatment is light chlorination at the pumping station and filtration through activated carbon at the laboratory. No residual chlorine was detected even in unfiltered tap water. A detailed analysis of St. Andrews town water by Thomas (1960), includes the following values: residue on evaporation 32 mg/l; sulphates 3.9 mg/l; and chlorides 4.0 mg/l. The laboratory water was similar to that analysed by Thomas, except for higher calcium content and higher pH, both caused by $Ca(OH)_2$ dissolved from an asbestos-cement pipeline (Sprague, 1964). The total hardness of laboratory water throughout this work was 20 mg/l as $CaCO_3$, and 15 mg/l of this was due to calcium. Total alkalinity was 12 mg/l as $CaCO_3$. The concrete pipe raised the pH of water from approximately neutral to pH 10. For the bulk of experiments, pre-treatment of water by aeration brought pH down to 7.3, with the range during acclimation and experiments limited to 7.1–7.5. A few experiments made use of the high pH to show the modifying effect on toxicity of zinc, and fish for these tests were acclimated at pH 9.5. Nominal test-temperatures were 17, 15, and 5°C, while actual average temperatures were not different by more than 0.2°, and none of the individual readings deviated by more than 1.0°C.

Fish were measured to the fork of the tail after experiments. The fish used in the group of tests at different pH values averaged 8.5 cm length, with a range of 6.4–11.7 cm. These fish had been collected in an unpolluted part of the Miramichi River, N.B., and held in the laboratory for at least 6 weeks at 15–17°C and pH 9.5, until tests were carried out during September 1961. All other experiments were done from October to December of 1962, using fish of average length 14.3 cm and range 10.2–19.5 cm. These had been reared in a hatchery, then held at St. Andrews for 4 months at 11–18°C and pH 7.3–7.6.

Fish were thus held under the same general conditions as prevailed during experiments, and were acclimated to the exact experimental temperature for at least 6 days beforehand. For those fish to be used in tests at 5°C, holding temperature was lowered from 14 to 5°C during 12 days, then kept at 5° for another 7 days. Mortality of fish during holding was 5% or less in all groups. Fish were fed chopped liver daily during holding and acclimation, and in experiments lasting for more than 2 days were offered about half the usual amount of food, with remnants siphoned out an hour later. Fish had been exposed for 2 or 3 hr to sublethal concentrations of zinc and/or copper some weeks or months before they were used in lethal tests. It is unlikely that this had any effect on their resistance through acclimation to metals. Some of the largest fish were of smolt or post-smolt size, but this did not seem to change their resistance.

Inspection of results of individual experiments, and chi-square tests applied to various tabulations by size and order of death, failed to show any effect of size on resistance-time.

A constant flow apparatus was used, all components of which were glass, fiberglass, Plexiglas, or Tygon plastic tubing. Thermoregulators and quartz-covered heaters in a chamber of the water supply line maintained constant temperatures. Water was cooled for 5°C experiments by passing it through a glass heat-exchanger in an ice-bath. Diffused air kept dissolved oxygen near saturation in the test-tanks. Stock solutions of copper and zinc sulphate were held on a shaking machine to keep them homogeneous, and desired flows of stock solutions were maintained by small peristaltic tubing-pumps or by another type with plastic working parts. The added solution mixed in a funnel with the dilution water, which in most experiments flowed at 300 ml/min. This flow equalled in 1.5 hr the holding volume of 27 l in the test tanks. The experiments done in 1961 on modifying effects of pH used a flow of 1.9 l/min, which equalled the holding volume of 64 l in about $\frac{1}{2}$ hr.

The concentration of metal in the experimental water was determined at least once a day, and the average of all determinations represented a test. Deviations of individual determinations from the mean value averaged only 4.7%. None was greater than 18% and these larger deviations were not critical in the particular experiments involved. Measured zinc concentrations agreed with the theoretical or "intended" concentrations, but measured concentrations of copper were about 80% of the theoretical strengths. Since the copper was in low concentrations, small but relatively significant amounts may have been adsorbed on the walls of the apparatus, been taken up by the fish, or reacted with soluble organic matter and colloids in the water. This *organic* copper is considered undissociated (Riley, 1939), probably has no direct lethal effect on fish, and is not measured by our sodium diethyldithiocarbamate procedure (Sprague and Carson, MS, 1963). All values are given in micrograms of metal (Cu or Zn) per liter of solution. This unit has the disadvantage that some concentrations of zinc must be given in thousands, but it seems less awkward than frequent use of the third decimal place of mg/l.

The measure of resistance used in this work is time to 50% mortality (LT50) at a given concentration of metal. Five fish were customarily used for each LT50. Their individual resistance-times were determined by inspections after suitable periods of exposure, which increased more or less logarithmically. The criterion of death was absence of respiratory movements and lack of response to mechanical stimulation. Survival for 7 days was considered to represent indefinitely long survival, signifying no direct lethal effect of the metals. Results were analysed by the methods of Litchfield (1949), which start with a plot of percentage mortalities against resistance-times on logarithmic-probability paper. After fitting a line to the points by eye the LT50 is read from the graph. The number of animals and a slope factor are used to obtain 95% confidence limits for the LT50.

RESULTS

INCIPIENT LETHAL LEVELS

Results of all lethal tests with copper and zinc are given in Table I, and Fig. 1 shows those for near-neutral pH and 15–17°C. Use of logarithmic scales allows the sets of points to be represented by straight lines. All results in Fig. 1 were obtained in 1962, except for two tests with zinc. The LT50 for 1920 μg/l of zinc was obtained at pH 7.0–7.2 which is about the same as the pH used in 1962 experiments, while the LT50 for 5500 μg/l of zinc was obtained at pH 7.9. Neither this, nor the 2° difference in test-temperature, nor the different source of fish seems to have had much effect, and the LT50's fit reasonably well with

TABLE I. Details of experiments on mortality of young salmon exposed to copper and zinc solutions. Tests at 17°C were carried out in September 1961 after acclimation of fish to 17° and pH 9.5. Tests at 15° were done in October–December 1962 after acclimation to the experimental levels of temperature and pH. The laboratory water contained 2 μg/l of copper and 3 μg/l of zinc.

Temp. (C)	pH	Metal (μg/l) Copper	Metal (μg/l) Zinc	LT50 (*hr*) (and 95% confidence limits)	Comments
15°	7.1–7.5	260	3	12.2 (10.6, 14.0)	
"	" "	140	3	16.2 (13.1, 20.1)	
"	" "	78	3	25.2 (20.2, 31.5)	20% survival at 139 hr
"	" "	55	3	32.4 (28.9, 36.3)	
"	" "	49	3	27.8 (20.3, 38.1)	20% survival at 160 hr
"	" "	46	3	0% dead in 190 hr	
"	" "	25	3	20% dead in 164 hr	Died of fungus on injured eye
15°	7.1–7.5	2	4160	4.6 (4.0, 5.3)	
"	" "	2	2260	7.2 (5.8, 8.9)	
"	" "	2	1350	11.5 (8.4, 15.8)	
"	" "	2	690	25.2 (19.7, 32.3)	40% survival at 254 hr
"	" "	2	650	24.4 (15.3, 39.0)	40% survival at 160 hr
"	" "	2	510	0% dead in 113 hr	
"	" "	2	388	0% dead in 240 hr	
"	" "	2	3	0% dead in 763 hr	Control experiment
17°	7.9	2	5520	5.0 (3.5, 7.2)	Zn^{++} = 4600 μg/l*
"	8.2–8.4	2	4150	11.8 (7.8, 17.9)	Zn^{++} = 1130 – 460 μg/l*
"	8.6–8.9	2	3360	25% dead in 280 hr	Zn^{++} = 180 – 44 μg/l*
"	7.0–7.2	2	1940	10.3 (7.1, 14.9)	Zn^{++} = 1940 μg/l*
"	8.8–9.2	2	1920	20% dead in 217 hr	Zn^{++} = 70 – 12 μg/l*
"	8.9–9.2	2	1310	0% dead in 90 hr	Zn^{++} = 44 – 12 μg/l*
"	8.9–9.3	2	271	0% dead in 960 hr	Zn^{++} = 44 – 7 μg/l*
5°	7.1–7.5	2	2890	28.0 (23.9, 32.8)	
"	" "	2	886	0% dead in 209 hr	
15°	7.1–7.5	109	1250	5.3 (4.5, 6.3)	
"	" "	41	430	11.6 (9.9, 13.6)	

* These are the amounts of dissolved zinc in the experimental water at the specified values of pH, according to O'Connor (MS, 1961). The additional zinc measured in the water in most experiments was apparently in suspension. In the experiment at pH 7.0–7.2, 50,000 μg/l of zinc or more would have dissolved in the water.

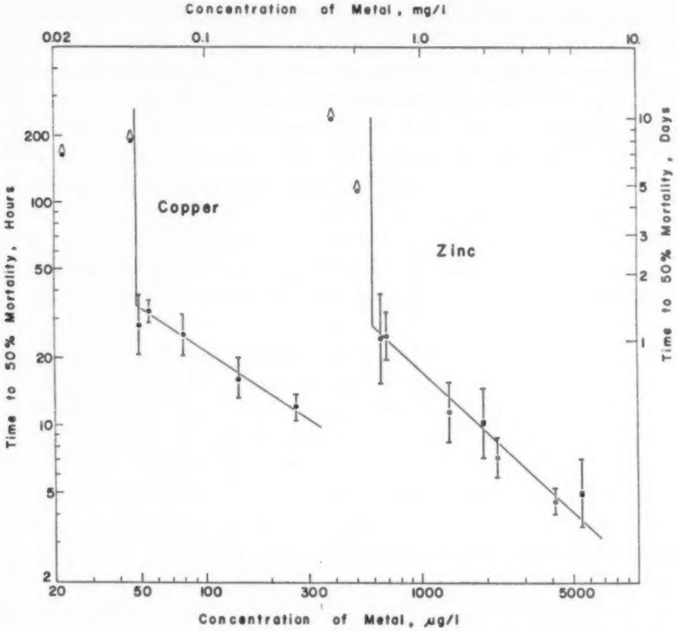

FIG. 1. Median mortality-times of young Atlantic salmon exposed to solutions of copper and zinc. Vertical bars indicate 95% confidence limits. The straight lines fitted to the points break and run parallel to the time-axis at the *incipient lethal levels*. The experimental water had a total hardness of 20 mg/l as $CaCO_3$, temperature of 15°C, and pH 7.1–7.5, except for 2 zinc tests indicated by black squares. These were at 17° and somewhat different pH values.

the other points, a straight line passing through the 95% confidence limits of all. Mortality did not proceed beyond 60% in tests at 650 and 690 µg/l of zinc, and these must be very close to non-lethal concentrations. Similarly there was 20% survival in the tests at 49 and 78 µg/l of copper. The longest LT50 in any test was 32 hr. Control experiments proceeded throughout the tests in both years, with no mortality during 31 and 40 days.

It seems from Fig. 1 that there is very sharp differentiation between lethal and non-lethal concentrations of each metal, and the line relating concentration to survival-time apparently breaks and runs parallel to the time-axis. The critical concentration of toxicant has been called by various names, but a suitable one has been given by Fry (1947). His term is part of a general scheme of classifying environmental factors, can be applied to any environmental identity, and has been used in lethal temperature and lethal oxygen work. The *incipient lethal level* is defined as that level of the environmental identity beyond which the organism can no longer live for an indefinite period of time. The term is abbreviated in this paper to ILL. From Fig. 1, the ILL for copper must be very close to 48 µg/l, and for zinc approximately 600 µg/l.

Having determined these values, any concentrations of copper or zinc may be expressed as a proportion or fraction of the incipient lethal level following the concept of Lloyd (1961b). The results for Atlantic salmon have been replotted this way in Fig. 2. For any given fraction of the ILL, zinc causes death more rapidly than copper.

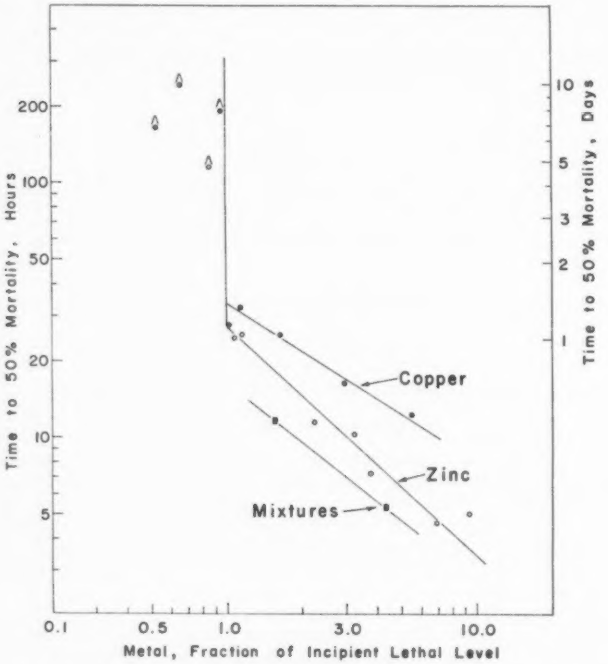

FIG. 2. Comparison of median mortality-times of young Atlantic salmon exposed to solutions of copper, zinc, and mixtures. Concentrations are expressed as fractions of the incipient lethal levels, those for copper and zinc being added together for the 2 experiments with mixtures.

COMBINED ACTION OF COPPER AND ZINC

Two tests were carried out with mixtures of copper and zinc, each metal contributing about half of the toxicity. The LT50's have been represented in Fig. 2 by adding the fraction of ILL for copper to the fraction of ILL for zinc in the mixture. The fish died more rapidly than would be expected from the total fraction of ILL. The LT50's for the mixtures are only about half as long as would be the case if they fell midway between LT50's for copper and zinc singly. The incipient lethal level for mixtures was not determined.

ZINC TOXICITY IN ALKALINE WATER

Several tests were conducted at higher pH values, utilizing the alkaline laboratory water described earlier. Aeration and the addition of zinc sulphate made the test-water somewhat less alkaline than pH 9.5 used to acclimate the fish.

It is known that solubility of a zinc salt decreases greatly as pH rises above 7.0, and in this experimental water, the amount of zinc which will dissolve is governed by the solubility of zinc hydroxide (O'Connor, MS, 1961). Theoretical solubilities of zinc taken from O'Connor's calculations have been included in Table I for the group of tests at elevated pH levels. Most of the concentrations of zinc measured in the water are in excess of the solubilities, presumably because the chemical test measured not only dissolved zinc, but also zinc in suspension as fine particles.

It has already been mentioned that the test of resistance to a measured 5520 μg/l of zinc at pH 7.9 agreed reasonably well with the set of results at pH 7.3. However, if the theoretical value of 4600 μg/l of dissolved zinc is used as the test-concentration, the result fits even better, with the LT50 in Fig. 1 being moved to the left, closer to the line fitting all the points. The survival-time in 4150 μg/l of measured zinc at pH 8.2–8.4 does not agree with the survival-time for the same concentration at pH 7.3. The LT50 was 11.8 hr instead of 4.6, significantly different at the 5% level. However, if we adopt the maximum value for soluble zinc during this test, which was 1130 μg/l, the LT50 agrees well with that of 11.5 hr for the similar concentration of 1350 μg/l of zinc at pH 7.3, and is in reasonable agreement with the line fitting all points in Fig. 1, which passes within the 95% confidence limits of the LT50. For 3 other tests at raised pH values, there was no mortality, or less than 50% mortality at measured zinc concentrations which apparently should have been lethal. Here again the result is explained by assuming that only soluble zinc is toxic, since the maximum solubilities in these tests were all below the incipient lethal level.

ZINC TOXICITY AT LOWER TEMPERATURES

Two tests at 5°C were carried out, and it is believed that the fish were acclimated to approximately this temperature. In a test at 2890 μg/l of zinc, the LT50 was 28 hr, compared to less than 7 hr which would be expected at 15°C. This is an increase in survival-time of a little more than 4 times for the 10° change in temperature. Another test at 890 μg/l was survived by all fish for more than 8 days. Apparently this concentration was non-lethal, although it would have caused 50% mortality in less than a day at 15°C. Therefore the incipient lethal level for zinc changed from about 600 μg/l at 15° to 900 μg/l or more at 5°C.

DISCUSSION

Incipient lethal levels of 48 μg/l of copper and 600 μg/l of zinc for Atlantic salmon show reasonable agreement with other recent work. For rainbow trout, another member of the genus *Salmo*, Lloyd and Herbert (1962, fig. 3) showed that copper and zinc ILL's are 50 and 700 μg/l in water of 20 mg/l hardness. Liepolt and Weber (1958) also used rainbow trout in water of the same hardness, and their results for copper indicate an ILL in the vicinity of 100 μg/l, twice as high as results given above. This lesser toxicity probably resulted from the lower test-temperature of 10°C, since Liepolt and Weber seem to have found a difference of about the same magnitude between copper ILL's at 10 and 15°C in their tests in hard water. Maloney and Palmer (1956) working in soft water of 41 mg/l hardness, found that 49 μg/l of copper caused 50% mortality of fathead minnows in 4 days, a value which is probably close to the ILL. The estimate for fathead minnows by Tarzwell and Henderson (1960) is very close — 50 μg/l of copper in water of 20 mg/l hardness.

Reasonable agreement between all results given above indicates that much of the heterogeneity in the older work summarized by Doudoroff and Katz (1953) resulted from differences in experimental conditions such as water hardness or species of fish. For example, recent work shows that bluegills resist 4 or 5 times as much metal as salmon, the ILL's in soft water being approximately 210 μg/l of copper (Tarzwell and Henderson, 1960) and 2500 μg/l of zinc (Cairns and Scheier, 1957).

In the present work, a straight-line relation was shown between the logarithm of survival-time and the logarithm of metal concentration, and the ILL is distinctly marked by a break in the line. This is convenient but may not be the case for all species. Although copper LT50's obtained by Liepolt and Weber (1958) for rainbow trout in soft water show a straight-line relation when plotted on logarithmic paper, Lloyd (1960, 1961b) found this only in very soft water, while LT50's in harder water approached the ILL in a gradual curve. As a related factor, these authors found LT50's as long as 55 and 75 hr, whereas Atlantic salmon apparently sustained 50% mortality within 33 hr or not at all.

Potentiation of toxicity in mixtures of copper and zinc cut survival-times of Atlantic salmon in about half — a phenomenon which has been previously observed for these metals (Doudoroff and Katz, 1953). Lloyd (1961b) found potentiation only for soft water and very high concentrations of metals. Below 7.0 times the ILL, he found that toxicities of copper and zinc singly were equal when expressed as fractions of ILL, and were merely additive in mixtures, contrary to the present findings with salmon. Furthermore, he determined that the ILL for mixtures was determined by simple addition. The present work did not ascertain for salmon whether the ILL of mixtures of copper and zinc can be predicted from the separate ILL's, but it is hoped to investigate this at some future time.

The apparent reduction in toxicity of zinc sulphate at pH 7.9–9.3 is explained simply by assuming that lethal action is exerted only by zinc in solution, not by suspended zinc. Whether this is the correct explanation is not known. Lloyd (1960) presents data which indicate that suspended zinc is toxic. The same general conclusion would hold for Lloyd's data if his values for concentrations of zinc in solution were replaced by theoretical values determined from solubility of zinc hydroxide, which is likely the governing factor for solubility (J. T. O'Connor, personal communication). There does not seem to be any way of reconciling that conclusion with the present findings, which indicate that only dissolved zinc is toxic.

It is difficult to determine what significance should be attached to results of Liepolt and Weber (1958) showing increased toxicity of copper at pH 6.0–6.4 compared to pH 7.6. They give "added" concentrations of copper, not amounts determined by analysis, so it is possible that at the higher pH some of the copper was no longer present either in solution or suspension, but had precipitated out of the water.

A change in temperature from 15° to 5° increased survival-time of Atlantic salmon by about 4 times in one test with zinc. This is somewhat more than the 2 or 3 times usually expected for a change of 10°. Results from other work vary, and are not strictly comparable since all are for temperatures above 10°. Liepolt and Weber (1958) show a 3-fold change for a mere 5° rise from 10° to 15°, but temperature acclimation was not described and could have changed the effect considerably. Plotting the data of Cairns and Scheier (1957) shows relatively little difference in copper-resistance of bluegills for 18° and 30°, after thorough acclimation. Rainbow trout show a 2- or 3-fold change in resistance-times at lethal levels of zinc between 11.5–21.5°, but there is no great change in the ILL (Lloyd, 1960). The present work at a lower range of temperatures shows that a change from 15° to 5° raises the incipient lethal level of zinc for Atlantic salmon by a factor of at least 1.5.

SOMMAIRE

1. On a déterminé dans une eau douce (dureté 20 mg/l comme $CaCO_3$) la toxicité des sulfates de cuivre et de zinc pour les saumoneaux de l'Atlantique (*Salmo salar* Linné), au stade parr.
2. La relation entre la concentration des solutions de métal et le temps de survivance des saumoneaux est représentée par une droite sur des échelles logarithmiques. Il existe un seuil des concentrations fatales, au-dessous duquel il n'y a pas de mortalité.
3. Les seuils des concentrations fatales à une température de 15°C et pH 7.1–7.5 étaient 48 μg/l de cuivre et 600 μg/l de zinc.
4. Pour comparer l'action des deux sels, on a exprimé les concentrations en multiples des seuils. Les solutions de zinc ont causé la mortalité plus rapidement que les solutions de cuivre.
5. Dans un mélange de solutions de cuivre et de zinc, le taux de mortalité des saumoneaux était deux fois plus élevé qu'on s'y attendait en supposant que l'action toxique des deux métaux était additive.
6. Les temps de survie en solutions de zinc ont été prolongés à pH 7.9–9.3, alors que le métal est moins soluble qu'à neutralité. Cette variation dans le temps de survie pourrait être expliquée en supposant que le zinc en solution est toxique, mais non pas en suspension.
7. Le temps de survie était quatre fois plus long à 5° qu'à 15°C dans la même concentration de zinc. Le seuil de concentration fatale pour 5°C était 900 μg/l au moins, à comparer à 600 μg/l à 15°C. Les saumoneaux étaient acclimatés à chacune des températures avant de les soumettre à l'action toxique des différentes solutions.

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Movements of Young Atlantic Salmon in a Small Stream[1]

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ABSTRACT

The locations of tagged parr and fin-clipped fry were observed from summer through early winter in a 1000-ft (328-m) study area of a small coastal stream. Tagged parr were usually found in or near places of original capture which are herein designated as homes. Parr whose homes were in pools, which appeared to be as suitable habitats as riffles, and those in adjacent riffles appeared to stay in their respective habitats. Some returned to their homes after having been moved as much as 700 ft (213 m) upriver or downriver. Fry were most numerous in shallow riffles and appeared to remain within small areas of the stream during summer but moved into parr habitats, the pools and deep riffles in autumn.

INTRODUCTION

Atlantic salmon (*Salmo salar* L.) spend 2 or more years in streams before descending as smolts to sea. While in fresh water, salmon parr are often observed defending territories in flowing water (Kalleberg, 1958; Keenleyside and Yamamoto, 1962) but few observations have been reported of their movements outside of these territories. In a review of the subject of restricted movements of fish populations, Gerking (1959) lists 4 salmonid species believed to have limited movements within streams. Carlin (personal communication, cited in Kalleberg, 1958) observed that juvenile Atlantic salmon have restricted movements. The purpose of the present study was to show the extent of movement of salmon parr and fry in a small stream during summer and early winter and to determine what factors influence this movement.

STUDY AREA

This work was done from August to December 1962 in the Waweig River of the St. Croix River system in southern New Brunswick. The Waweig is a small coastal stream about 8 miles long. Observations were made in a study area (Fig. 1) 1000 ft long and approximately 1.5 miles above the head of the estuary. This area contains a variety of habitats with water flow varying from shallow riffles (as little as 4 inches of water flowing over fine gravel) to deep pools (relatively still or slowly flowing water from 2 to 4 ft deep). Bottom characteristics range from fine gravel through rubble to boulders and bed rock.

Good numbers of fry (young-of-the-year) and parr (age 1+ and 2+) were found throughout the study area. In late October several spawning salmon and redds were found in the study area. Most spawning appeared to be in another

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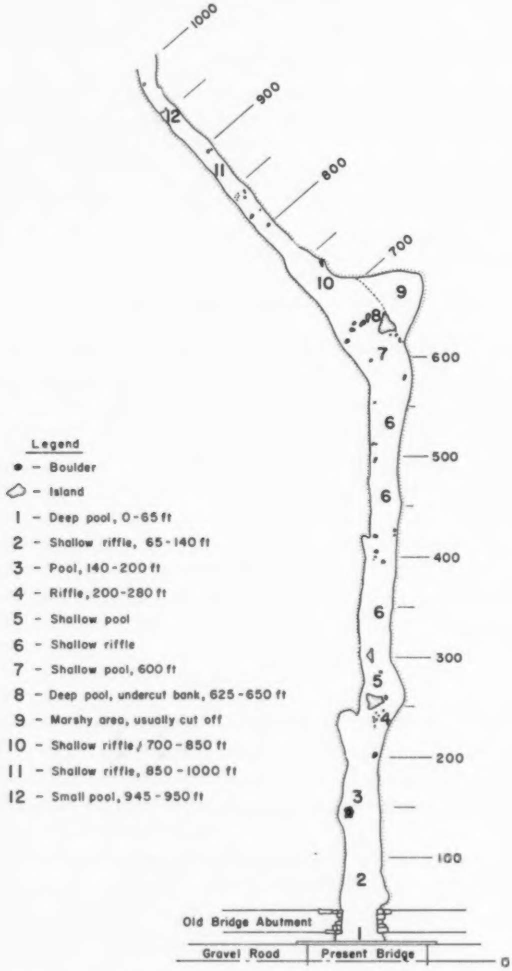
area about one-half mile upstream. Other fishes taken in the study area were brook trout (*Salvelinus fontinalis*), ninespine stickleback (***Pungitius pungitius***), white sucker (*Catostomus commersoni*), American eel (***Anguilla rostrata***) and several species of cyprinids. Although only two brook trout were taken, the other species were numerous.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0
12
11
10
9
8
7
6
6
6
5
4
3
2
1
Legend
● - Boulder
◇ - Island
1 - Deep pool, 0-65 ft
2 - Shallow riffle, 65-140 ft
3 - Pool, 140-200 ft
4 - Riffle, 200-280 ft
5 - Shallow pool
6 - Shallow riffle
7 - Shallow pool, 600 ft
8 - Deep pool, undercut bank, 625-650 ft
9 - Marshy area, usually cut off
10 - Shallow riffle / 700-850 ft
11 - Shallow riffle, 850-1000 ft
12 - Small pool, 945-950 ft
Old Bridge Abutment
Gravel Road
Present Bridge

FIG. 1. Map of Waweig River study area. The study area was measured and marked off at 50-ft intervals as shown. Various features of the stream are indicated.

METHODS

A 2-man, 15-ft hand seine was used to capture fry and parr initially for tagging and subsequently to determine their whereabouts. An electrofishing apparatus employing 500 volts D.C. was used during the final observation in December. On 2 occasions a snorkel, faceplate and flippers were used to aid in the observations of tagged parr.

Stream sections of various lengths were blocked off with barrier nets for initial capture of parr. For convenience in describing subsequent movements of individual parr, these sections of river are herein designated as homes. From the observations discussed below, it was concluded that many homes so designated were too large and could have been defined much more closely.

Only parr were tagged. Tags were made of fine, white polyethylene monofilament in 5-cm lengths in which overhand knots were tied in line in various combinations so that their number and placement represented numbers. Attachment was made by passing 2 strands of filament through the musculature at the base of the dorsal fin. These were tied on either side of the fish and the length of material bearing knots was left to trail out on one side.

Fry from 3 widely separated locations within the study area were distinctively marked by removing either the adipose or left or right pelvic fin.

Fish were held in a live car until they appeared to have recovered from marking and were able to swim against the current. Except for 25 parr which were moved various distances upriver and downriver, marked fish were released near places of original capture.

Locations of marked salmon were determined at various times between August and December. Freshets precluded intensive sampling of the whole area at regular intervals. However, 2 systematic, 4-day surveys were made shortly after fish were tagged and liberated. Intensive sampling was done on small portions of the study area on 18 other occasions. Places of recapture were defined as closely as possible to give data on distance moved from points of original capture.

RESULTS AND DISCUSSION

Movements of Parr

Data concerning capture of parr, their tagging, release and recapture are summarized in Table I and illustrated in Fig. 2. Of 63 parr tagged and released in their respective homes, 48 were recaptured from 1 to 4 times. Forty-five of these were subsequently caught or observed in or near their respective homes a total of 72 times. Recaptures were made from 2 to 123 days after release, over half of them after more than 20 days. Only 3 parr (No. 26, 40 and 42) were recaptured away from their homes; 2 of these (No. 26 and 42) were subsequently captured back in their homes.

All 13 of the parr tagged in the lower 60 ft of the riffle extending from 200–280 ft were recaptured at least once. All but one of 26 recaptures of these parr were made in the home riffle.

TABLE I. Movements of salmon parr tagged and liberated in their homes. These data are for 48 recaptures of 63 parr tagged and returned to their respective homes. Data for parr which were not recaptured are not included here, e.g. No. 5, 8, 12 . . . 62 and 63.

Tag number	Capture and liberation data		Recapture data		
	Date	Place (homes)[a]	Days elapsed	Place[a]	In or near home
		ft		*ft*	
1	Aug. 1	50–150	19	135	+
2	"	35–65	19	25–65	+
			40	25–65	+
3	"	50–150	19	135–180	+
			26	40–55	+
			40	25–65	+
4	"	50–150	2	140	+
6	Aug. 3	145–180	17	135–180	+
			42	170	+
7	"	145–180	17	135–180	+
9	"	145–180	42	195	+
10	"	145–180	17	135–180	+
			42	165	+
11	"	145–180	17	135–180	+
			42	190	+
13	"	145–180	17	135–180	+
			42	195	+
14	"	145–180	17	135–180	+
			42	185	+
15	"	145–180	17	135–180	+
			42	150	+
16	"	145–180	18	190	+
17	"	200–260	18	200–270	+
			52	270	+
18	"	200–260	45	200	+
19	"	200–260	42	250	+
			45	220	+
20	"	200–260	18	200–270	+
			41	230	+
			52	250	+
21	"	200–260	18	240	+
22	"	200–260	18	270	+
			41	260	+
			42	265	+
			52	275	+
23	"	200–260	52	275	+
			123	280	+
24	"	200–260	18	255	+
			45	210	+
25	"	200–260	45	245	+
			53	245	+

TABLE I. (continued)

Tag number	Capture and liberation data		Recapture data		
	Date	Place (homes)[a]	Days elapsed	Place[a]	In or near home
		ft		*ft*	
26	Aug. 3	200–260	38	25–65	–
			41	215	+
27	"	200–260	18	240	+
			45	270	+
			80	250	+
28	"	200–260	18	270	+
29	"	200–260	18	220–270	+
30	Aug. 6	285	16	285	+
31	"	400–450	16	410–465	+
			60	420	+
33	"	400–450	16	410–465	+
34	Aug. 7	465–500	16	470	+
35	"	465–500	15	420–465	+
37	"	465–500	16	470–500	+
38	"	630–650	16	625–650	+
			29	635–645	+
40	"	630–650	17	800–850	–
42	"	630–650	16	720	–
			29	635–645	+
43	"	630–650	16	625–650	+
			29	635–645	+
44	"	630–650	16	625–650	+
			29	635–645	+
45	"	630–650	16	625–650	+
			29	635–645	+
46	"	630–650	16	625–650	+
47	"	780	16	720	+
48	"	830–840	17	800	+
49	"	830–840	20	840	+
51	"	830–840	17	800–850	+
52	"	830–840	17	825	+
53	Aug. 9	910	43	890	+
54	"	950–965	18	965	+
			27	965	+
56	"	950–965	27	965	+
61	Aug. 27	10–15	14	10–15	+

[a] Places refer to specific locations within the study area which was measured from 0 to 1000 ft (Fig. 1).

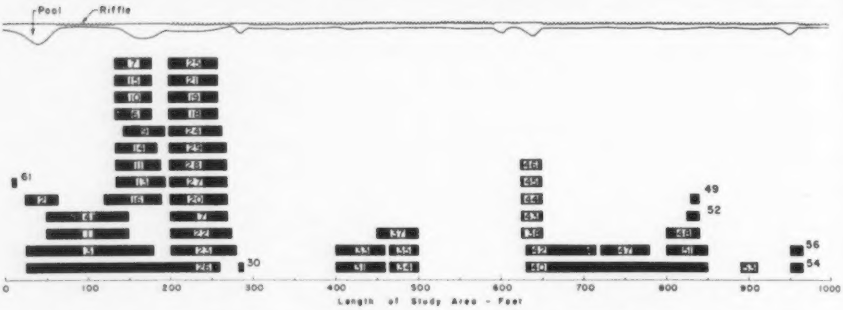

FIG. 2. Observed distribution of tagged parr in the study area. Each horizontal bar represents the maximum length of the river over which a tagged parr was observed at original capture and recapture(s). Data from Table I. Note locations of pools and riffles as indicated along top of figure.

Initially, it was assumed that seining activities caused fish to move considerably. Later it was observed that parr in riffles do not move very far when disturbed but retreat to nearby hiding places. After some experience, it was learned that particular individuals could be sought close to specific boulders or bushes. Thus, in many cases, points of original capture and recapture could have been pin-pointed and the stream sections designated as homes (Table I) could have been greatly reduced. For example, the home of parr No. 22 was assumed to be within the 200–260 ft marks of the study area. At four recaptures over 52 days, this fish was always taken within an area only 15 ft long.

MOVEMENT BETWEEN POOLS AND RIFFLES

A striking observation from this study was that, in general, parr whose homes were in riffles were recaptured only there, and those from pools were recaptured in their pools even when these different habitats were adjacent. This is best illustrated by the data in Table I concerning the parr (No. 6–16) in a pool and those (No. 17–29) in an adjacent riffle. Only one fish (No. 26) was found on different occasions in both of these habitats. It is not known if this close association with habitat was maintained during severe freshets, since seining was not done at such times.

MOVEMENT IN RELATION TO FLUCTUATING WATER LEVEL

Data obtained during the period of lowest water indicate that some individuals moved from shallow riffles to nearby pools when the water level fell. Only 3 such observations were made, probably because water remained unusually high during the study.

The movements of fish, No. 26, 40 and 42, appear to have been in response to changing water levels as follows: The home of parr No. 26 was the riffle at 200–280 ft. When the water level reached its seasonal low, this fish was observed in the pool at 0–65 ft. Following a freshet, with the depth of the riffle restored, it was recaptured again in its home. Parr No. 40 and 42 were taken originally in a deep pool when the water level was near its seasonal low. They were taken next, following a freshet, in riffle areas well above the place of original capture. No. 42 returned subsequently to the pool and was taken there during a second period of low water. These 3 fish apparently moved from riffles to pools when the water level fell and returned to riffles when the water level rose. In a river in which hydroelectric power plants caused severe fluctuations in water level, Lindroth (1955) found that brown trout (*Salmo trutta*) populations are not stable and probably move constantly with respect to the shore line.

POOLS AS HABITATS FOR PARR

In many streams salmon parr are often observed living in riffles. In the Waweig they were abundant both in pools and riffles. Large pools in the study area at 0–65 ft and 140–200 ft, taken together, held as many parr as the well-populated riffle at 200–280 ft. It is concluded that pools may be considered as useful habitats for young salmon. Observations in a Prince Edward Island stream support this view (J. W. Saunders, personal communication). There it was observed that during years with low population densities of brook trout, salmon parr were found in both riffles and pools, but in years when trout were abundant, salmon parr were found principally in riffles. The near absence of brook trout from the Waweig study area may have permitted salmon parr to live in pools which might otherwise have been occupied by trout.

HOMING OF DISPLACED PARR

Of 25 parr tagged and released various distances upriver and downriver from their homes, 9 were observed from 14 to 39 days later (Table II). Seven of the 9 were recaptured in their homes, one was caught where it was released and the other about 100 ft from point of release. Four of the displaced parr were eventually recovered in their original homes from 200 to 660 ft upstream from points of release. Three were recovered in their homes from 210 to 770 ft downstream from points of release. These data indicate a tendency for at least some of the translocated parr to reoccupy their homes. This supports the previous conclusion that parr are strongly attached to particular, small areas of the river.

MOVEMENTS OF FRY

Fry were numerous throughout the study area. In August, three groups of fry were distinctively marked and released near their respective homes, 123 between 50 and 200 ft, 135 between 450 and 670 ft, and 48 between 850 and 1000 ft. From the three respective groups, 54, 46 and 16 fry were recaptured during the next 2 months. Most recaptures were made within the limits of original capture. Those in adjacent areas were usually only a few feet from these limits. Of 116 recaptures of the 306 fry marked, only one had moved far outside the area in which it was first observed.

The observed tendency of young salmon to remain within narrow limits may have an important application in the management of this species. Elson (1962) reports that heavy plantings of hatchery-reared salmon fry in the Pollett

TABLE II. Movements of tagged salmon parr after experimental displacement upriver or downriver. Data for the recaptures only of the 25 parr tagged and displaced.

Tag number	Capture and liberation data			Recapture data	
	Date	Place captured[a] (home)	Place released[a]	Days elapsed	Place recaptured[a]
		ft	*ft*		*ft*
D 1	Aug. 27	900–950	500	39	390
D 8	"	800–820	650	25	800
D 9	"	800–820	500	24	800
D 12	"	825–840	225	18	250
D 13	"	825–840	650	28	830
				29	830
				39	830
D 14	"	825–840	225	29	885
D 18	"	40–55	820	14	35–65
D 21	"	40–55	245	14	35
D 22	"	40–55	650	14	25–65
				17	40

[a] Places refer to specific locations within the study area which was measured from 0 to 1000 ft (Fig. 1).

River, New Brunswick, which was deficient in young salmon, resulted in limited dispersal upstream and downstream.

Movement of fry to protected areas in fall

In summer, fry were found mainly in shallow riffle areas. In autumn, they became less numerous in such places but were found in greater numbers in the pools and deeper riffles already occupied by parr. During the final observation, by electrofishing in December, the water was high and cold (about 0.1° C). No fry were taken in the shallow riffle between 65 and 140 ft where they were numerous in August. Five fry originally marked in this area were taken in the pool just below and the deep riffle above. Fry and parr were taken only from protected areas such as overhung banks, among large rocks and under boulders. It is doubtful that hand-seining would have been nearly as effective as electrofishing in collecting such fish under these conditions.

Scarcity of Parr in Autumn

Sections where good catches of parr were made in summer yielded few or none during repeated seinings after late September. Of those captured, most were in or near their homes. One factor contributing to these reduced catches may have been that parr were hiding among the gravel and could not be easily dislodged. Few parr were seen in open areas where they were observed earlier in the season. Most fish seined in the autumn were taken by vigorously stirring up the gravel a few feet above the seine. Lindroth (1955 and personal communication) observed that salmon parr in winter may be found in the gravel below the surface of the stream bed.

Another factor contributing to poor catches may have been the movement of parr to other areas of the river. This could have been owing to lack of suitable areas for wintering or may have been in response to the presence of large, spawning salmon from the sea. Jones and King (1948) observed that male parr move upstream to areas where adults are spawning. Saunders (1960) reports that from 10–20% of the resident population of salmon parr in Ellerslie Brook, Prince Edward Island, moved downstream in autumn and these migrants were predominantly ripe males. Meister (1962) observed in a small coastal stream in Maine that many more salmon moved downstream in autumn as parr than in spring as smolts.

SUMMARY

This was a study of the movements of Atlantic salmon fry and parr within a small coastal stream during summer and early winter. In a 1000-ft study area, parr were serially tagged and fry were marked by fin-clipping. Their subsequent whereabouts were determined at frequent intervals.

1. Individual parr were seldom observed away from particular small areas which were designated as their homes.
2. Parr from pools and those from adjacent riffles appeared to stay in these respective habitats unless the water fell to low levels. There is some evidence that they leave riffles and move to nearby pools during periods of low water.
3. Pools appeared to be as acceptable habitats for parr as riffles which are usually considered as preferred habitats for parr.
4. Some parr returned to their homes after having been moved upriver or downriver as much as 700 ft.
5. Like parr, salmon fry remained within small areas during summer. Most were found in shallow riffles in summer. In the autumn they appeared to move into the summer parr habitat, the deep riffles and pools.
6. Parr appeared to be scarce in the study area in autumn. They may have hidden among the gravel from which it was difficult to dislodge them or they may have moved out of the study area. Although few parr were recaptured in autumn, those few were taken, as in summer, in or near their homes.

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We are grateful to Dr P. F. Elson who offered valuable suggestions for both the field work and preparation of this report. We thank our co-workers, Drs C. J. Kerswill, M. W. Smith, J. B. Sprague and Mr J. W. Saunders, who gave helpful suggestions for presenting the findings. Mr P. W. G. McMullon helped with the illustrations and Miss Marion Haley prepared the manuscript.

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Ultraviolet Irradiation of Circulating Refrigerated Fish Storage Brines[1]

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ABSTRACT

Irradiation of refrigerated brines with ultraviolet radiation was effective in controlling bacterial development in the brine during the post-mortem storage of lingcod (*Ophiodon elongatus*) and sockeye salmon (*Oncorhynchus nerka*). The bacteriological and organoleptic quality of the fish was not improved by continuous or intermittent exposure of brines to ultraviolet radiation. Irradiation of highly contaminated turbid brines resulted in the killing of a large percentage of the initial bacterial population. Intermittent or continuous irradiation of brines produced off-odours in the brine and in the stored fish. Discolouration developed in the irradiated brine and on exposed surfaces of the stored fish.

INTRODUCTION

The method of transporting fish in refrigerated sea water and of subsequently holding certain species of fish in this medium on shore has expanded extensively during the past 10 years. Roach *et al.* (1961) have reviewed investigations on the engineering, bacteriological and biochemical problems involved in storage and transport of fish in refrigerated sea water.

Although the germicidal activity of ultraviolet radiation (UVR) has been studied by many investigators concerned with the control of bacterial contamination in various types of food, limited information is available on the effect of UVR on fish spoilage microorganisms. Tarr *et al.* (1938) studied the effect of exposure of fresh fish fillets to UVR and found that irradiated fillets had lower bacterial counts, better flavour, and fresher odour after storage at 1.7°C (35°F) than non-irradiated fillets. Young *et al.* (1939) tested the effect of UVR in controlling the bacterial contamination of brines used for the brining of fish prior to smoking, and it was shown that brining solutions could be sterilized by UVR. Roach *et al.* (1958) constructed an experimental ultraviolet water sterilizer to study the effect of UVR on the control of bacterial contamination in circulating refrigerated fresh fish storage brines; results obtained with it in a limited number of trials indicated that significant bacterial kills could be obtained with UVR of low intensity. Rowan (1956) carried out experiments on the sterilization of sea water by ultraviolet light. Using the spore-former, *Clostridium welchii*, as the indicator organism, the sterilizer achieved a 92–100% kill.

The present investigation was undertaken to ascertain the germicidal effect of UVR against fish spoilage bacteria in refrigerated sea water, and also to assess the resultant effect of such treatment on quality of fish held therein.

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MATERIALS AND METHODS

Two pilot-plant-scale circulating refrigerated brine systems were used in this study to test the effectiveness of UVR in controlling or inhibiting development of bacteria in refrigerated brines. Each refrigerated system was equipped with a chiller. The chillers employed were of the type designed by Harrison and Roach (1957), which have been used extensively in refrigerated sea water installations on fishing vessels and packers. For the present investigations, insulated coolers with a total water capacity of 8 gal were used as tanks for the storage of fish in refrigerated brines. For convenience a 3% sodium chloride–water solution was used as the refrigerated fish storage medium in place of sea water. The temperature of the refrigerated brine was maintained between 0 and −1°C (32 and 30.2°F).

A commercial germicidal ultraviolet (UV) water sterilizer (Aquafine Corporation, Los Angeles, Calif.) was connected between the discharge of the chiller and the fish-storage tank of one brine system. The remaining system was used as a control tank unit in which a sterilizer was not installed. In both systems

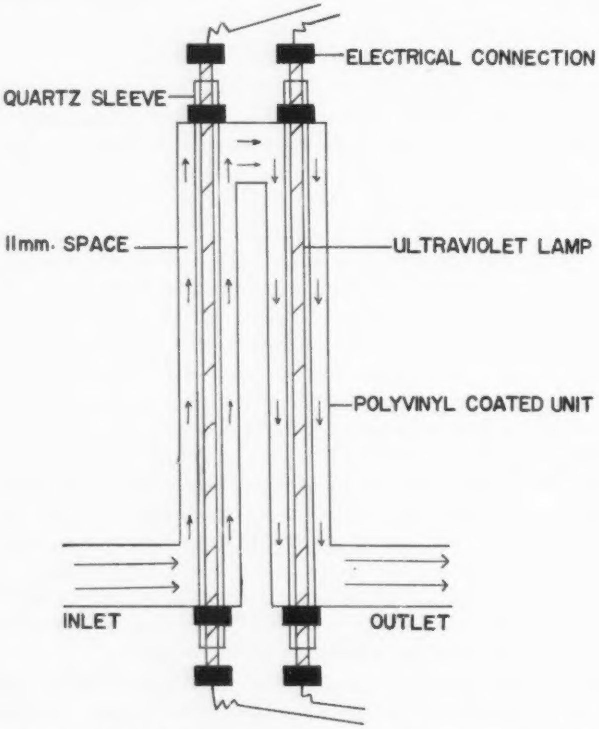

FIG. 1. Schematic diagram of polyvinyl-coated two-lamp ultraviolet unit used in the experimental irradiation of refrigerated brine.

the brine was recirculated by a pump from the storage tank through the chiller and returned to the tank. The UVR of the sterilizer, at 2537 A, was generated by 2 Westinghouse 39-watt, 115-volt germicidal lamps which were encased in separate quartz UV transmitting sleeves. The UV output of each lamp was rated at 13.8 total watts and 120 microwatts/sq cm at one meter from the lamp. The quartz sleeves were used in this refrigerated system to maintain a constant output of UVR since UV lamps operate erratically at temperatures below 12.7°C (55°F). Each quartz-sleeve-encased lamp was situated in one side of an H-shaped polyvinyl-coated (PVC) water circulating system (Fig. 1). The space between the outer surface of the quartz sleeve and inner surface of the PVC tubing for water circulation and irradiation was 11 mm. The circulating brine was pumped from the chiller to the PVC sterilizer in which system the brine passed over the quartz sleeves of each lamp where irradiation of brine was accomplished before it was returned to the storage tank.

CONTINUOUS IRRADIATION

To study the effect of continuous UVR on refrigerated brine contamination and on fish quality, two experiments were carried out on the storage of lingcod (*Ophiodon elongatus*) in brine. The brine circulation rate through the systems in this experimental series was 160 gal/hr. In Experiment 1, fresh dressed lingcod were stored in the irradiated and non-irradiated tanks. Each tank contained approximately 5 gal of brine and sufficient fish to produce a 1:2 ratio of fish to brine. At intervals, viable bacterial counts and organoleptic tests were carried out on both brine and fish. Similar methods were employed in Experiment 2, with the exception that fresh lingcod fillets rather than fresh dressed lingcod were stored in the brines.

INTERMITTENT IRRADIATION

To test the efficacy of intermittent irradiation of brines on the quality of stored sockeye salmon (*Oncorhynchus nerka*), experiments were carried out on the storage of fresh whole sockeye salmon in irradiated and non-irradiated refrigerated brines. The brine in each experiment was circulated at a rate of 320 gal/hr. In Experiment 3, salmon were stored in the respective brines for 8 days. Irradiation exposure times were for periods of 60–180 min in the morning and for 60 min in the afternoon every 24 hr. Experiment 4 consisted of storage of salmon for 10 days in the respective brines and irradiation of the brine under test for 120 min in the morning and for 60 min in the afternoon every 24 hr. In these last two experiments the value of intermittent irradiation of brines was assessed by the methods outlined in the first experiments.

At the conclusion of each experiment the germicidal activity of UVR was evaluated by exposing turbid and contaminated control brines to radiation for periods from 15 to 120 min. Tests for viable bacteria remaining in the brine were made after each exposure period.

For the determination of viable bacterial counts on brine samples, duplicate 10-ml aliquots were used from each tank, and appropriate dilutions were prepared from each sample in preparation of 3% (W/V) sodium chloride-Bacto nutrient agar plates. The plates were incubated at 25°C (77°F) for 72 hr. A Quebec colony counter was used in the determination of the number of colonies per plate.

To assess the bacteriological quality of stored fish or fillets, portions of fish approximately 1.5 cm square were cut aseptically from fish or fillets. The skin of whole or dressed fish was not removed from the portions. Duplicate samples of respective portions were used for viable bacterial count determinations. Forty grams of cut portions was added to 360 ml of sterile 3% (W/V) sodium chloride solution contained in a quart Mason jar and subsequently shaken on a reciprocal shaker for 5 min at 260 oscillations per min. Appropriate dilutions were made from each original fish sample dilution in preparation of agar plates. The plates were incubated at 25°C for 72 hr.

RESULTS AND DISCUSSION

Continuous UV irradiation of brine during storage of dressed lingcod and lingcod fillets was found to be effective in retarding bacterial development (Fig. 2). It will be noted from the graphs of viable bacterial counts of brine samples that in each experiment a definite progressive increase in bacteria occurred in the non-irradiated brines, whereas in the irradiated brines bacterial populations either decreased or were maintained at reasonably constant levels. After 6 days of storage (representative example) irradiated brine from each experiment was found to contain 99% less viable bacteria than the non-irradiated brine.

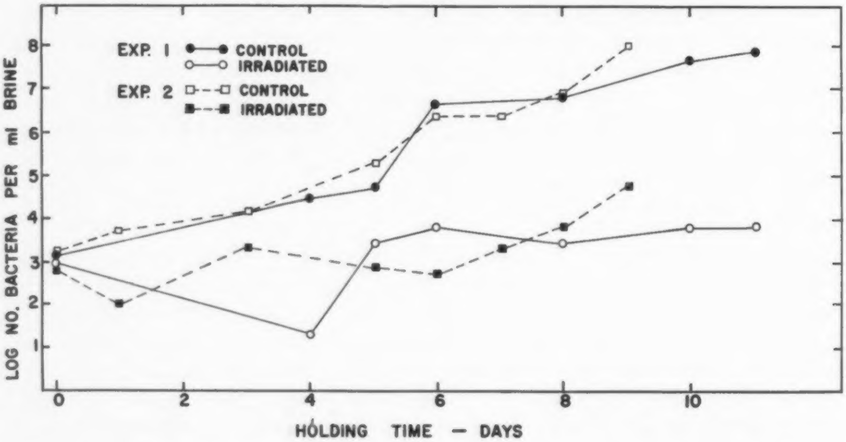

FIG. 2. Viable bacterial counts of ultraviolet irradiated (continuous) and non-irradiated brines: Exp. 1 — storage of dressed lingcod; Exp. 2 — storage of lingcod fillets.

Figure 3 presents in graphic form the results of Experiments 3 and 4 on the effect of intermittent irradiation on the control of bacterial increase in refrigerated brine during storage of whole sockeye salmon. Viable bacterial counts of

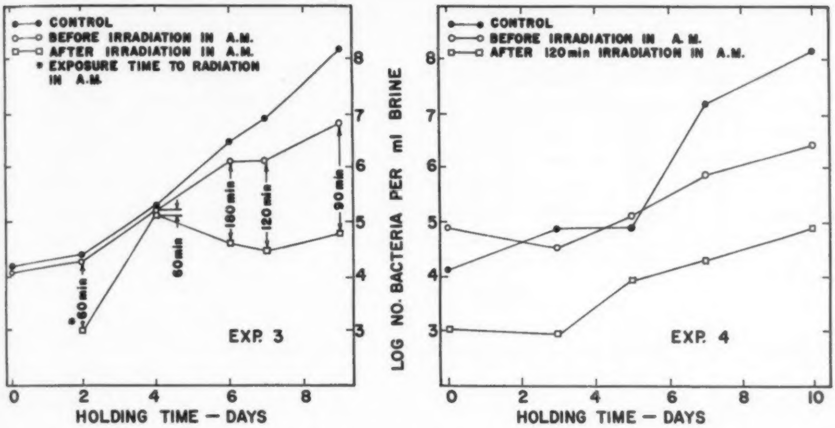

FIG. 3. Viable bacterial counts of ultraviolet irradiated and non-irradiated brines during storage of whole sockeye salmon: Exp. 3 — intermittent irradiation of brine for 60 min in P.M. and for varying periods in A.M. Exp. 4 — intermittent irradiation of brine for 60 min in P.M. and for 120 min in A.M.

irradiated and non-irradiated brines after 6 days storage (Exp. 3) showed that there were 2.5 times more viable bacteria in the non-irradiated brine than in the irradiated sample taken before irradiation in the morning. Following exposure to UVR for 180 min in the morning, the bacterial count of the irradiated brine was reduced by 97% which increased the viable bacteria ratio of the non-irradiated brine to irradiated brine from 2.5:1 to 80:1. After 9 days storage irradiated brine had 96% fewer viable bacteria before morning irradiation than non-irradiated brine, which at this time had 173 × 10^6 bacteria per ml. After 90 min exposure to UVR the viable bacterial count in irradiated brine was reduced by 97%. Similar results were obtained from viable bacterial studies which were made on irradiated and non-irradiated brines in Experiment 4 (Fig. 3). Although results obtained from the 4 series of experiments on the effect of UVR on the control of bacterial development in refrigerated brines indicate that continuous or intermittent irradiation of brine is effective in controlling bacterial increase, the results of organoleptic evaluation indicated the development of undesirable side effects in irradiated brines. Irradiated brines had a light brown discolouration and also a characteristic off-odour. The stored fish or fillets were also tainted and had a slight brown discolouration on exposed surfaces. The undesirable colour changes and off-odours were presumably due to the effect of UVR on dispersed lipids or proteins. Brown discolourations could be partly due to formation of methemoglobin or metmyoglobin.

The results of viable bacterial counts on fish or fillets which were stored in irradiated or non-irradiated brines are presented in Fig. 4. The results indicate that UV irradiation of brines under the experimental conditions was not effective in retarding bacterial growth in stored fish. It may be noted that the fish

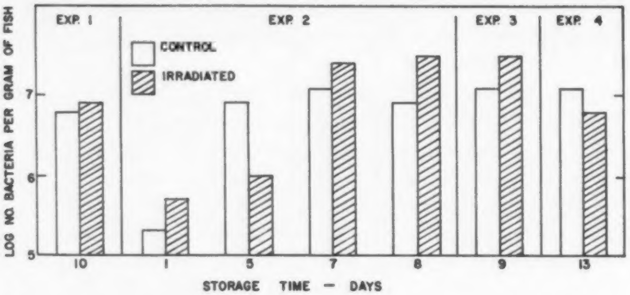

FIG. 4. Viable bacterial counts of fish samples from ultraviolet irradiated and non-irradiated brines during four experimental series.

samples from irradiated brine had, in the majority of tests, higher viable bacterial counts than fish samples from non-irradiated brine.

As reported by Wood (1961) the degree of sterilization of water by UVR depends upon the total exposure, which is a function of the intensity of the light source, and on the optical density and rate of circulation of water. A particular dose of radiation may be obtained by means of a short exposure of high intensity or by a long exposure of low intensity. A series of experiments was conducted on turbid, contaminated, non-irradiated brines at the conclusion of each experiment as described above to observe the germicidal effect of irradiation after different periods of exposure to UVR. The results are illustrated graphically in Fig. 5. Since the brines were quite turbid a 100% bacterial kill was not obtained after exposure to UVR for periods as long as 120 min. After 30 min exposure in the majority of tests a bacterial kill of from 97.0 to 99.9% was obtained with the one exception of Experiment 4, in which only a 42% kill was obtained after 30 min exposure. A 99 to 99.9% bacterial kill was obtained in brines from the 4 experiments after exposure to UVR for 60 min. During the

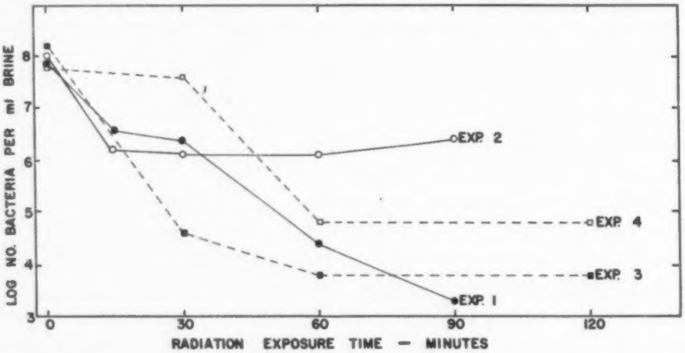

FIG. 5. Viable bacterial counts of turbid, non-irradiated (control) brines from four experiments after different exposure times to ultraviolet radiation.

UVR exposure trials on brine it was observed that slight off-odours and slight discolouration developed in the brines after 30 min exposure.

It is concluded that under the conditions used in this investigation, the treatment of refrigerated brines with UVR is very effective in preventing bacterial development in the brines, however, the bacterial quality of stored fish is not improved by such treatment. Irradiation also induced the development of off-odours and colour changes in brines; the odour of stored fish was altered and the exposed surfaces were slightly discoloured. Turbid, highly contaminated refrigerated brines are almost "sterilized" by UVR after a reasonable period of exposure.

ACKNOWLEDGMENT

The authors acknowledge with thanks the assistance of Mr E. G. Baker of this laboratory in the installation of refrigerated brine systems.

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The Origin of Cold High-Salinity Water in Foxe Basin[1]

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ABSTRACT

During the 1955 cruise of HMCS *Labrador* to the Canadian Arctic an unusual mass of cold saline water was discovered in Foxe Basin and Channel. Temperatures as low as −1.90°C were observed in association with salinities of the order of 33.75 and 34.00‰.

An explanation of the origin of this water is presented and compared with additional studies conducted by the author in the same area in 1956.

INTRODUCTION

In 1955 and 1956, the Atlantic Oceanographic Group of the Fisheries Research Board of Canada undertook two extensive oceanographic surveys in the Eastern Archipelago from HMCS *Labrador*. In 1955, surveys were conducted in Hudson Strait and Hudson Bay in June and October, and in Foxe Basin in July, August, September and October. In 1956, the work was repeated in Hudson Strait, Foxe Basin and Foxe Channel.

Quite distinct from the general temperature and salinity conditions described by Campbell and Collin (MS, 1956) and Campbell (MS, 1958; 1959), the 1955 survey revealed an extensive mass of cold high-salinity water in the western areas of Foxe Basin and Channel. Temperatures were observed below −1.80°C and salinities above 33.75‰. In the following year, temperatures and salinities were observed to be less extreme in Foxe Basin but still significantly different from the oceanographic conditions in Hudson Bay and Strait.

The likelihood of such cold saline conditions existing in Foxe Basin or Foxe Channel was deduced earlier by Hachey (1954) on the evidence of his investigations in Hudson Bay in 1930. Hachey (1931) found waters with temperatures less than −1.80°C and salinities ranging from 32.21 to 33.24‰ at depths below 50 m, and reasoned that such water did not originate in Hudson Bay but entered the area from Hudson Strait or Foxe Channel.

FOXE BASIN AND CHANNEL

Foxe Basin (Fig. 1) is a shallow, featureless inland sea surrounded mainly by precambrian and palaeozoic land formations. Most of the basin is palaeozoic in origin in contrast to Foxe Channel which is precambrian. Basin depths average less than 50 fathoms with the shallowest regions located along the eastern perimeter and the deepest off Melville Peninsula and Fury and Hecla Straits (Fig. 2).

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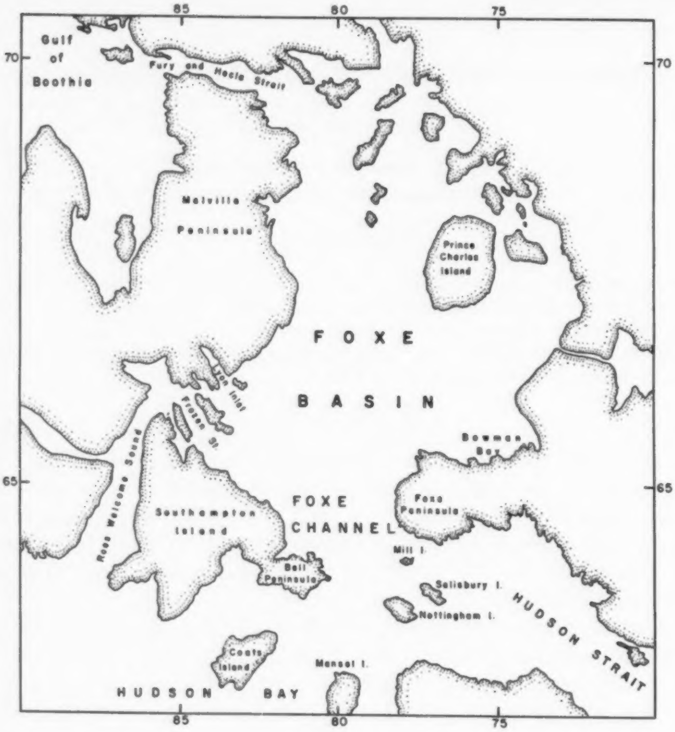
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80
75
70
65
Gulf of Boothia
Fury and Hecla Strait
Melville Peninsula
Prince Charles Island
FOXE
BASIN
Lyon Inlet
Frozen St.
Roes Welcome Sound
Southampton Island
FOXE CHANNEL
Bowman Bay
Foxe Peninsula
Mill I.
Bell Peninsula
Salisbury I.
Nottingham I.
HUDSON STRAIT
Coats Island
Mansel I.
HUDSON BAY

FIG. 1. Foxe Basin.

Foxe Channel is similar in many respects to Hudson Strait, both of which are characterized by single deep channels extending throughout their lengths. These are continuous through the island chain from Nottingham Island to Foxe Peninsula but terminate either in Frozen Strait or Lyon Inlet. The northern slope of Foxe Channel gives way to the plateau-like plain of Foxe Basin, while in the south a submerged ridge of 100 fathoms or less cuts across the channel between Bell Peninsula, Southampton Island and Nottingham Island.

GENERAL FEATURES OF THE OCEANOGRAPHY

The circulation features a strong, deep southerly flow along the western perimeter of the basin and channel deviating only at its southern limit where it is deflected into Hudson Strait by the ridge off Bell Peninsula. Most of the deep water enters Hudson Strait between Nottingham and Salisbury Islands but part of the subsurface water swings around Bell Peninsula and enters Hudson Bay (Campbell, MS, 1959). Strong peripheral tidal currents which probably give rise to the counter-clockwise gyre in central Foxe Basin occur from Foxe Peninsula to Bowman Bay.

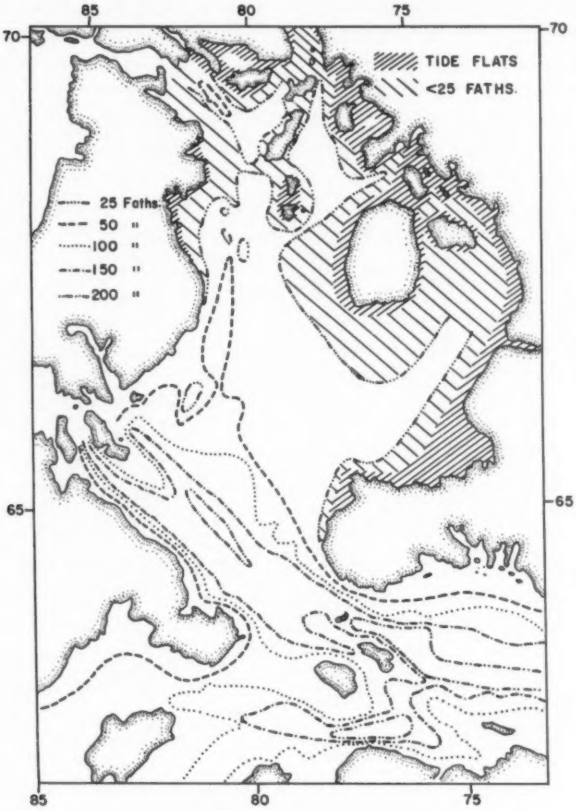


FIG. 2. Bottom topography and tide flats, Foxe Basin.

A deep inflowing current enters Foxe Channel from Hudson Strait and extends northward, at least as far as the sill of the basin. Possibly some of this water feeds into the central gyre but most of it must be returned in the outflowing southerly current on the western side of the channel.

For the purpose of characterizing the cold high saline water some of the environmental features and T-S characteristics for 1955 and 1956 are noted. In general the range of temperature is small even for the surface waters, the lowest temperature recorded being −1.77°C and the highest 3.11°C. The average summer temperature for the basin and channel was 0.3°C. Surface salinities occasionally fell as low as 4–20‰, reflecting local dilutions of melt water and river run-off, but by late summer and early autumn surface salinities levelled off at 29–32‰. In 1955, average monthly subsurface temperatures and salinities (20–50 m) in northern Foxe Basin varied from −1.2°C and 32.0‰ in July, to −0.5°C and 30.9‰ by October.

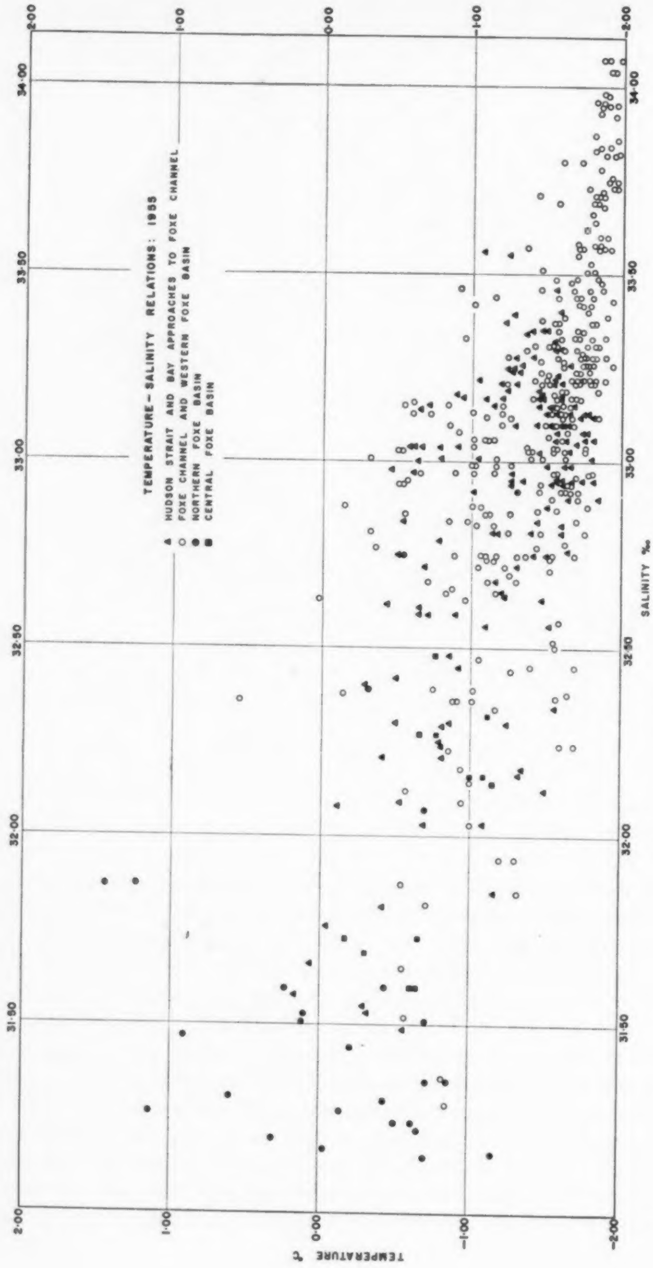


FIG. 3. Temperature-salinity characteristics, 1955.

Temperatures and salinities in Foxe Channel ranged from −1.5°C and 33.2‰ in early summer to −0.9°C and 32.4‰ in the autumn. The northern area of the basin exhibited a wider fluctuation and range of temperature and salinity than either Foxe Channel or western Hudson Strait (Fig. 3). The fluctuations are attributed to periodic discharges of ice into this area from Fury and Hecla Straits, heating and cooling, local sources of melt water and river run-off. In central Foxe Basin temperatures varied from approximately 0°C to a lower limit of −1.4°C and salinities 32.5 to 31.3‰. The water at each station would be considered well mixed since at most stations a single T-S point characterized the entire water column. In this area the variation in the T-S relationships is largely a function of geographic location.

In Foxe Channel and western Foxe Basin the majority of the subsurface observations fell within the limits of −0.5 and −1.8°C and 32.5 and 33.5‰ but some subsurface temperatures were observed as low as −1.97°C coincident with salinities of 33.75‰ and better.

DISTRIBUTION OF COLD SALINE WATER

1955

In Foxe Basin the temperature and salinity environment of −1.80 to −1.97°C and 33.75 and 34.07‰, respectively, is quite distinctive in comparison to the marine climate of Hudson Bay and Strait (Fig. 3). Figure 4 illustrates the vertical and longitudinal distribution of the water along the eastern side of Southampton Island and Melville Peninsula. For the most part the water is

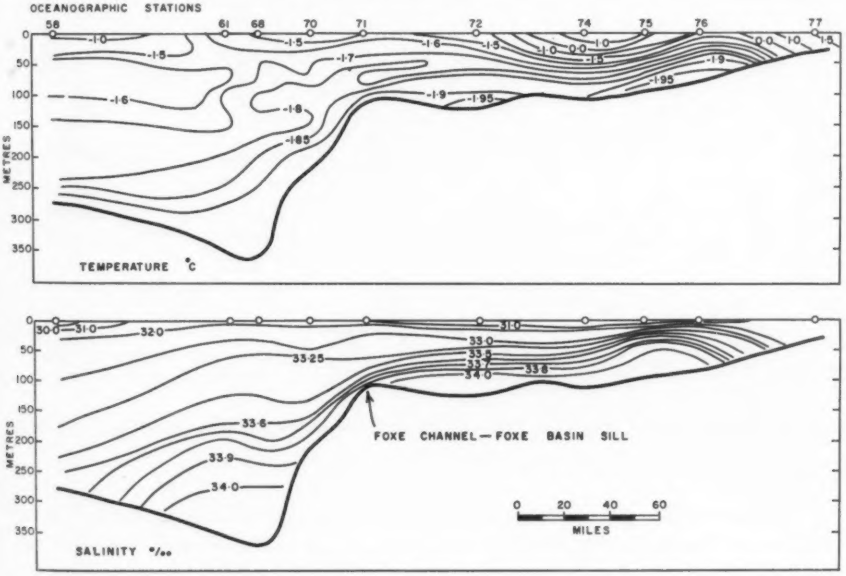


FIG. 4. Longitudinal profile of temperature and salinity through Foxe Channel and Basin, 1955.

confined to the bottom but at several points, Station 76 and others (not shown), the mass of cold saline water extends upwards to 50 m depth.

Figure 5 illustrates the geographical distribution and range of minimum temperatures and maximum salinities from June through to October 1955. The Figure clearly illustrates a concentration of cold saline water on the western side of the basin. In northern and central Foxe Basin minimum temperatures varied from −0.5 to only −1.5°C and −1.0 to −1.4°C, respectively. Lower salinities also persisted in northern Foxe Basin throughout the summer period reflecting in part *in-situ* melting of local ice and that discharged through Fury and Hecla Strait. River run-off from northern Baffin Island and Melville Peninsula also tends to maintain a brackish water condition in this area.

In comparison to the conditions in Foxe Basin, minimum temperatures in Hudson Bay varied only between −1.25 and −1.75°C. Salinities of 33.0 and 33.5‰ appear to represent maximum seasonal values for June and July as evidenced by a subsequent decrease in salinity in the fall.

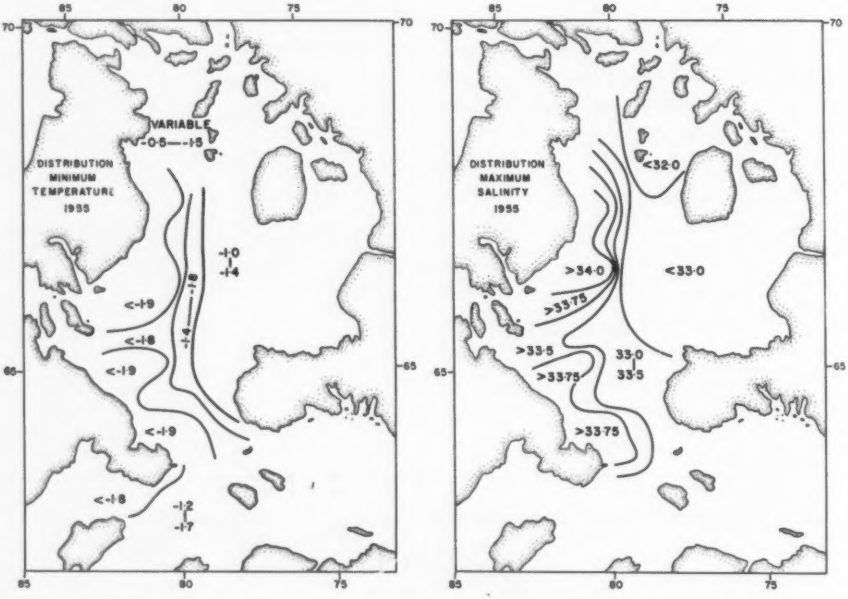


FIG. 5. Distribution of minimum temperature and maximum salinity, June–October, 1955.

1956

Similar studies were carried out in Foxe Basin and vicinity in the following year and it was quite evident from the outset of the observational program that marked marine climatic changes had occurred in the region. These effects are indexed by the T-S plots for the period July to September, 1956 (Fig. 6).

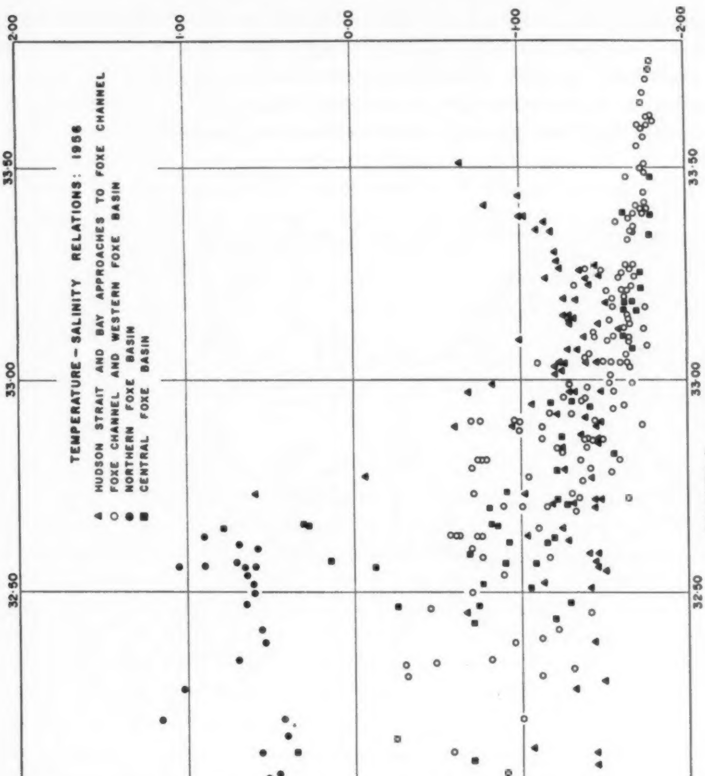
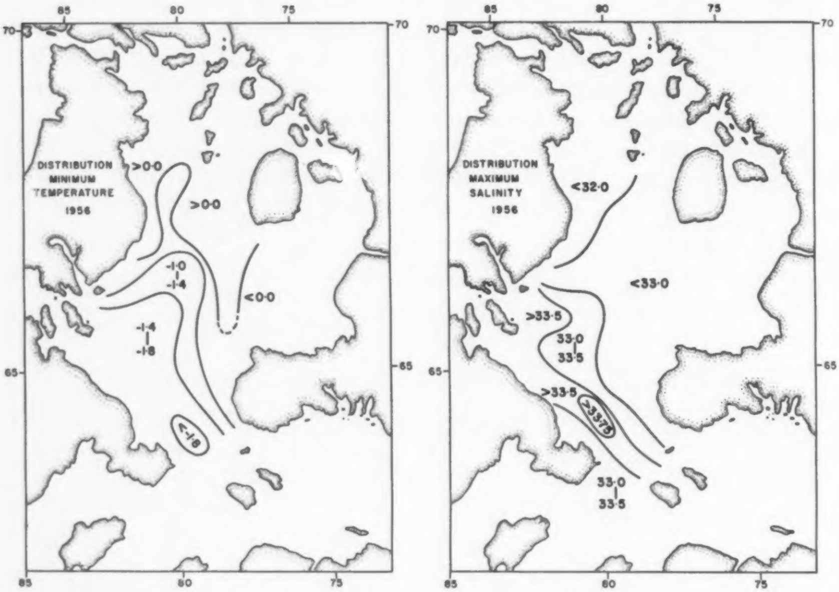


FIG. 6. Temperature-salinity characteristics, 1956.

In northern and central Foxe Basin minimum temperatures were only about 0°C while in western Foxe Channel they ranged from −1.4 to −1.8°C as compared to −1.8 and −1.9°C in 1955. The maximum salinity in 1956 was less than 33.75‰ but, despite these changes in the character of the water, a cold saline water mass could be identified (Fig. 6) and located in the same geographical areas as in 1955 (Fig. 7).


85
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DISTRIBUTION MINIMUM TEMPERATURE 1956
>0·0
>0·0
-1·0
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<0·0
-1·4
-1·8
<-1·8
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80
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DISTRIBUTION MAXIMUM SALINITY 1956
<32·0
<33·0
>33·5
33·0
33·5
>33·5
>33·75
33·0
33·5

FIG. 7. Distribution of minimum temperature and maximum salinity, July–September, 1956.

The confinement of the cold saline water to the western side of Foxe Channel and Basin appears to be most certainly related to depth but restricted geographically by turbulence and mixing. In areas such as the eastern side of Foxe Channel and central Foxe Basin large eddies are visible on the surface when the tide is running. Here the water is well mixed and essentially homogeneous from top to bottom, whereas in the regions where the cold saline water is found the water is distinctly stratified.

Observations taken in the southern area of the Gulf of Boothia in 1956 revealed minimum temperatures of −1.0 and −1.5°C and salinities of 29.0 and 32.7‰ from the surface to 150–200 m. Salinities of 33.7 and 34.0‰ but with temperatures of −1.0°C were observed in the northern section of the Gulf and in Prince Regent Inlet at depths of 275 m (Collin, MS, 1958). This water could only enter Foxe Basin if it passed over a sill of 75 m depth in Fury and Hecla Strait.

No trace of such cold water was found in Hudson Strait and Bay in June and October 1955 or in July and August 1956. Extreme temperatures were of the order of –1.7°C between 50–150 m with salinities only varying between 32.5 and 33.0‰ (Campbell, MS, 1958). Salinities greater than 34.0‰ were observed only at the mouth of Hudson Strait but with temperatures well over 0°C. In northern Hudson Bay, however, temperatures varied from 1.0°C at the surface to –1.5°C at 100 m, and –1.25°C below 200 m off Digges Island. Salinities in Hudson Bay appeared to be generally lower than Hudson Strait exhibiting values less than 31.0‰ at the surface and not greater than 33.3‰ at 300 m.

No data are available from Fury and Hecla Strait for the winter of 1954–55, but in 1955–56 a winter station of 50 m depth was regularly monitored off Igloolik by Grainger (1959). He reported that the minimum sea temperature was –1.75°C and maximum salinity 32.52‰ for the period of greatest ice thickness. Water conditions at this time were uniform throughout the water column.

FORMATION OF THE WATER

The factors contributing to the formation of this water are puzzling in themselves since, on the face of it, the general oceanographic and climatological conditions are not unique or peculiar to this part of the Arctic. In another study, this writer pointed out the oceanographic significance of the tidal flats in Foxe Basin which have no other parallel in the Canadian Arctic (Campbell and Collin, 1958). They cover an area of almost 6000 square miles or 10% of the area of the Basin (Fig. 2). In Bowman Bay for instance the intertidal zone is approximately 10 miles in breadth while in other regions it may extend seawards 2–5 miles. The tidal flats vary in character but for the most part consist of boulder-strewn beaches and broad, flat mud banks.

As mentioned earlier weather conditions are not any different in Foxe Basin than in neighbouring areas. However, the fact that a large portion of the sea bottom would be chilled in the winter on exposure to the air is significant and under severe weather conditions the intertidal muds would freeze any water trapped on the banks. This region, therefore, provides an additional cooling surface where repetitive processes of freezing and leaching of sea water can occur with the change of tide.

Sea water would certainly be chilled below its marine *in-situ* temperature on coming in contact with the exposed tidal flats and doubtless some bottom ice would form, causing an increase in the salinity. On an ebb tide some of the sea water would remain in poorly drained areas and freeze, producing a concentrated brine solution which could be chilled further without freezing. Drainage or seepage from the tidal flats would be slow and most of the resultant mixing with sea water would have to await the following change of tide. As long as suitable air temperatures prevailed the processes described could continue almost indefinitely, limited only by the amount of ice that can be stored on the tidal flats.

Frazil ice growth must also be considered as a possible contributing agent to the *in-situ* increase of salinity. All the necessary factors that contribute to the formation of ice in this manner exist in Foxe Basin, namely, turbulence, suspended particles, and open water.

Calculations based on an increase in salinity of 0.6‰ indicate that 8–10 times a heat loss would be required from the tidal flats than that necessary to form 3 m of undisturbed sea ice. This estimate is considered to be within reason since one could presume that the minimum area of surface radiation from stranded ice flows or boulders would be about 5 times greater than that of drifting sea ice.

DISCUSSION

The occurrence of the cold saline water in Foxe Basin and vicinity in 1955 and to a lesser extent in 1956 is well substantiated by the number of observations and T-S correlations. The results lend credence to Hachey's early conviction that such water likely forms in Foxe Basin (Hachey, 1954).

Subsequent surveys to Foxe Basin, however, have failed to reveal the recurrence of the cold saline water since 1955 and 1956. These surveys have been on the eastern side of the channel and well offshore from Melville Peninsula owing to heavy concentrations of ice on the western side. This may partly account for the lack of further evidence, but the dependence of the phenomenon on the relative severity of the winter cannot be ignored. Figure 8 is a plot of the deviation from normal air temperatures (°F) as recorded at Nottingham Island, Hudson Strait. The winter conditions for the years 1953–54 and 1954–55 were well below normal while those for 1955–56 were above normal. According to Bates (1958) this difference amounts to approximately 600–800 degree-days and, in terms of ice growth, this is approximately 7–10 inches less undisturbed ice growth for 1956 than in 1955. Since the process of formation of the water depends

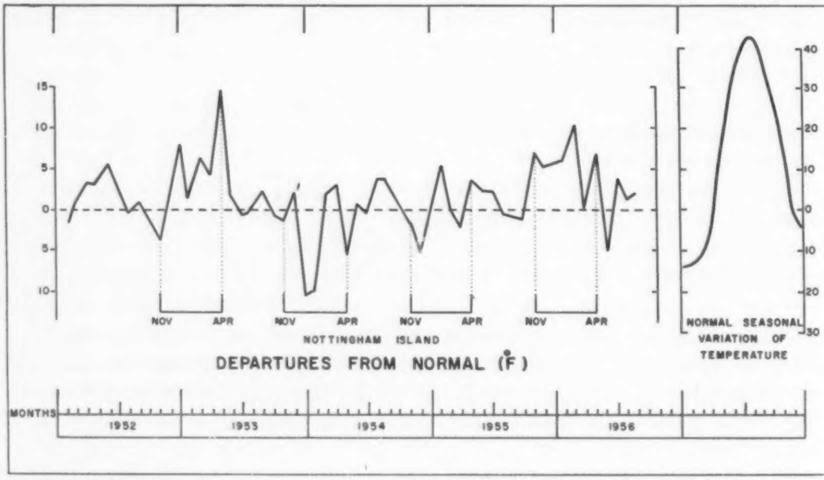

FIG. 8. Deviation from normal air temperature, Nottingham Island, Hudson Strait.

on the winter climatic conditions, it is not surprising that it is sporadic and variable. On the other hand one would expect to find some evidence of the water in parts of Foxe Basin in February and March even if the winter was relatively mild. In a severe winter, perhaps, conditions more extreme than those described for July and August 1955 might be expected.

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The author wishes to acknowledge the helpful assistance provided by the Commanding Officer, officers and men on the cruises of HMCS *Labrador* in 1955 and 1956, and as well various members of the Atlantic Oceanographic Group who participated in the cruises or offered helpful suggestions in preparation of this paper.

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Variations in the Growth of Atlantic Cod (*Gadus morhua* L.)[1,2]

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ABSTRACT

Growth rates of Atlantic cod vary both between populations in the same year and within a population over a series of years. The latter type of change is selected for detailed study, using an example found in the population resident in the western Gulf of St. Lawrence (ICNAF Division 4T). A real change in growth is verified by examining ageing methods, selectivity of sampling, tagging returns, and by measuring increments on the otoliths. Laboratory experiments showed that, when food supply is unlimited, food consumption increases with increasing temperature, and that growth increases with increasing food consumption. Direct correlation between temperature and growth changes is not established.

Cod growth changes in Division 4T are found to be the result of changes in abundance and availability of herring as food, plus changes in competition for food resulting from density changes in fish-eating members of the cod population.

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INTRODUCTION

MAINTENANCE of the biomass of a commercial fishery depends upon growth replacing fish removed by fishing operations and lost by natural causes. To explain changes in the fishery and to predict them, growth rates of fish are studied. This paper records and analyzes recent changes in growth rate in the cod population that has supported an otter-trawl fishery in the southwestern Gulf of St. Lawrence since 1947.

Environmental influences on growth have been widely discussed in the literature and these effects may be broken down into three groups. These include physical factors having some effect on the metabolism of the individual, factors related to availability of food, and the effect of density of the particular populations of fish in an area and their competition for food and space. Of the physical factors in the environment, one of the most frequently investigated is water temperature, probably because of its recognized influence on metabolism. For instance, LeCren (1958) found that year-to-year variations in growth of perch in the Windermere were positively correlated with the number of days that the temperature was over 14°C. Hempel (1958), investigating growth of plaice in the German Bight, indicated that increased growth was coincident with higher water temperatures in the feeding season. Molander and Molander-Swedmark (1957), keeping populations of the same species under controlled conditions in the aquarium, also found that growth was more rapid in high and slower in low temperatures. Brown's (1946a) tank experiments on trout showed a more complicated temperature relationship with growth maxima in two separated temperature ranges, 7–9°C and 16–19°C. This she found was caused by the varying values for appetite, maintenance and growth at different temperatures. Taylor (1958) has shown what appears to be a good correlation between surface water temperature, as an index of environmental temperature, and growth parameters for Atlantic cod.

However, examples can also be found of growth changes in fish where no temperature correlations were evident. A recent case was el-Zarka's (1959) study of growth changes in yellow perch in Saginaw Bay, where no correlation with temperature appeared.

The effects of density of population on growth have been considered in relation to food availability for individuals and the space factor. Recent reviews of the literature by Watt (1956) and Beverton and Holt (1957) indicate that it is the rule for fish to respond to changing density by altering their growth and that food availability is usually involved. In fact, LeCren's (1949) perch data are quoted as a notable exception to this rule. The space or crowding factor has been speculated upon in many works, but the actual evidence for it has come only from a few isolated experiments on fish in captivity. Willer (1929) is quoted as having noted a crowding factor in tank experiments which reduced the growth of fish even though a good food supply was available. More recently, Yoshihara (1952) has investigated the density factor and its apparent inhibiting effect on the growth of carp in ponds. Brown (1946b) found that very crowded and very uncrowded trout in tanks grew less well than those in densities of an intermediate range. Regarding the uncrowded individuals, she wrote that a certain amount of "social stimulation" seemed to be conducive to rapid growth.

COD GROWTH IN NATURE

SIZE AT AGE

METHODS. Growth of cod in different populations was estimated from age and length data from fish in samples of commercial landings. Samples of Canadian mainland landings have been taken routinely since 1946. These samples represent only the larger members of the cod population on the grounds since fishermen discard most of those under about 40 cm fork length. Sampling is usually complicated further by the practice of landing cod sorted into a number

TABLE I. Examples of observed ring counts and edge types on cod otoliths, and the resulting designation to age-groups.

Time of capture		Number of hyaline zones	Type and width of edge zone	Age-group
Quarter of year	Month			
I	Feb. Mar. Apr.	5	narrow hyaline	5
		5	wide hyaline	5
		5	narrow opaque	5
		5	wide opaque	5
II	May June July	5	narrow hyaline	5
		5	wide hyaline	5
		5	narrow opaque	5
		5	wide opaque	5
III	Aug. Sept. Oct.	5	narrow hyaline	4[a]
		5	wide hyaline	5 (4[a] in Oct.)
		5	narrow opaque	5
		5	wide opaque	5
IV	Nov. Dec. Jan.	5	narrow hyaline	4[a]
		5	wide hyaline	4[a]
		5	narrow opaque	5
		5	wide opaque	5

[a] In these cases it is assumed that a new hyaline zone has formed at the edge of the otolith before the assumed birthday of the fish in the following February. Therefore, it is not counted as a year-zone for age-group designation.

of market categories by sizes. This complication is eliminated by the port sampler who uses a system of weighting factors in order to randomize the sample of the catch obtained. The method used has been explained for haddock landings (Kohler, 1958) and operated in essentially the same way for cod.

Ages of Atlantic cod were determined by examining sacculus otoliths. Thompson (1943) used scales for ageing cod from the Newfoundland area and indicated that he had confidence in his readings up to about 8 years of age. Scales were also used by biologists working on cod in northeastern Atlantic areas (Graham, 1929a; Dannevig, 1933). However, the otolith is now believed to afford the best means of age determination of cod (e.g. Trout, 1958; Dannevig, 1956; Fleming, 1960). All ages used here have been determined from otoliths.

Earlier samples (1946–1954) of otoliths were removed from the fish and stored in vials in a 50% glycerin solution with enough thymol to prevent fungus growth. An investigation of storage methods in 1954 showed that the readability of the otoliths was not adversely affected by dry storage in coin envelopes. Since this method was much more convenient for both samplers and readers, it was adopted.

Age determination was carried out as follows: the otoliths were fractured across the longitudinal axis at a point near the interruption in the *sulcus acusticus*, the longitudinal groove on the convex side. The pieces were then embedded in a cube of modelling clay, the fracture surfaces being set flush with the horizontal upper surface of the cube. They were covered with 50% glycerin solution and then examined with the aid of a stereoscopic microscope (usually 9×) using reflected blue light. Hyaline and opaque zones in the otolith cross-section were counted to give the age of the fish. The interpretation of the zone at the edge of the otolith varies according to the season of capture of the fish, and makes a difference to age-group assignation (Table I). Interpretation of a hyaline edge in the third (Aug.–Oct.) and fourth (Nov.–Jan.) quarters of the sampling year may also vary slightly, depending on the particular population from which the cod came, but is normally handled as shown in the table.

VALIDITY OF AGE DETERMINATIONS. Methods of determining age should be verified when these are involved in the estimation of population parameters (Saetersdal, 1953; Graham, 1956). Three methods of verification of otolith age determinations were followed. They depend upon (1) annual change in the type of edge material found in the cross-section of the otolith, (2) the consistency of age data showing strong and weak year-classes in a population, and (3) the consistency of yearly production of annuli in the otoliths of fish in definable length-groups found by the Petersen method.

Variations in edge types of otoliths of cod from inshore Lockeport, N.S. (Fig. 1) landings are shown in Fig. 2. These landings are made up of fish from ICNAF Unit Area 4Xo (Fig. 1). The data include samples for 11 years, from 1948 to 1958, inclusive. They are broken down into months and the percentages of fish having otoliths with hyaline or opaque edges are indicated. The yearly cycle shows hyaline edges predominating from December to April and opaque edges in the months of June to September. In the intervening months

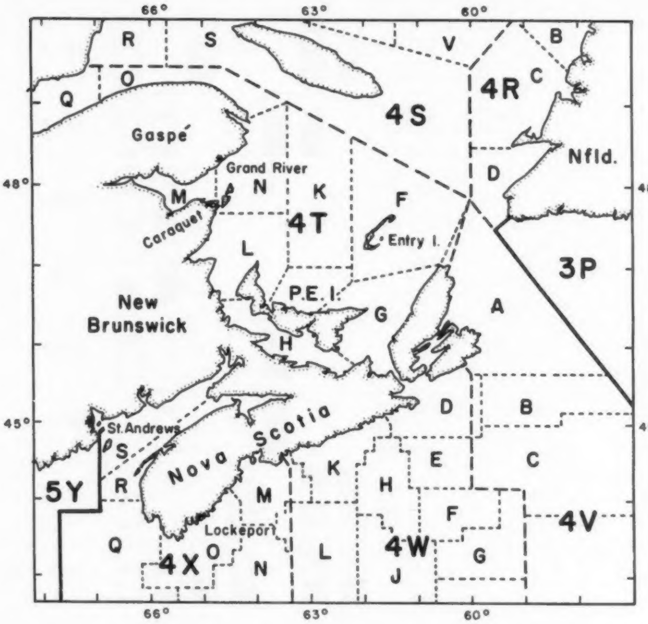

FIG. 1. Divisions and Unit Areas of ICNAF Subarea 4.

the dominant edge type varied from year to year. This pattern indicates the annual formation of one hyaline and one opaque zone on the periphery of the otolith. The same type of evidence has been used by Saetersdal (1953) for haddock and by Hickling (1933) for hake.

For the population of cod in the southwestern Gulf of St. Lawrence (ICNAF Division 4T) convincing information on otolith edge turnover is unavailable since weather conditions and sea ice often preclude sampling the same elements of the stock in both summer and winter. However, there is evidence of consistency of age readings from the way certain year-classes maintain their relative strengths over a series of sampling years. The data used here are taken from August–October commercial otter-trawl landings from the southwestern Gulf of St. Lawrence for the years 1949–1952 and 1955–1959. They are plotted in the form of percentage age frequencies in Fig. 3. The changing age structure of the population makes the identification of strong and weak year-classes easier in the 1955–1959 period than in the earlier period. In the early period, which coincided with the postwar buildup of the otter-trawl fishery in the area, a relatively large proportion of the cod landed was over 10 years of age. In this situation, where as many as 12 age-groups were contributing substantially to the fishery, only the 1941 year-class showed dominance in more than one sampling year. However, in the later period when the effects of the intensive fishery were reflected in the virtual disappearance from the landings

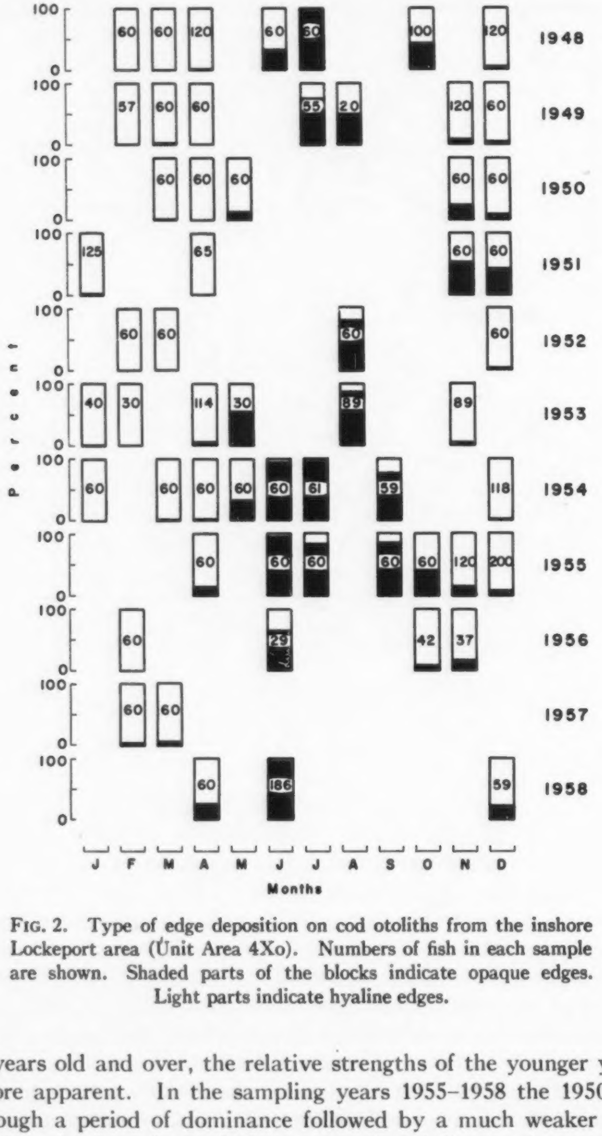

FIG. 2. Type of edge deposition on cod otoliths from the inshore Lockeport area (Unit Area 4Xo). Numbers of fish in each sample are shown. Shaded parts of the blocks indicate opaque edges. Light parts indicate hyaline edges.

of cod 10 years old and over, the relative strengths of the younger year-classes became more apparent. In the sampling years 1955–1958 the 1950 year-class passed through a period of dominance followed by a much weaker 1951 year-class. During the first three of these years the 1948 year-class was also consistently of medium strength, followed by a smaller 1949 year-class. Hile (1936), Dannevig (1933) and Graham (1929b) have all used such evidence of continued abundance and scarcity of year-classes to establish the general reliability of age determinations.

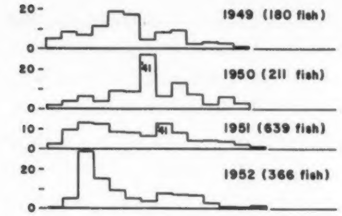

FIG. 3. Age composition of otter-trawl-caught cod landed in northern New Brunswick from ICNAF Division 4T (Aug.–Oct.).

In order to establish objective criteria for identifying the first annulus on the otoliths special collections of very young cod from the ICNAF Division 4T population were started in 1959. These young cod were located and caught using otter-trawling gear from the M.V. *Harengus*, an 84-ft research vessel used by the St. Andrews Station. Specifically, the trawls were a 40-ft shrimp trawl with a ¾-inch codend liner, and the usual No. 36 trawl used by the *Harengus* with the codend cover lined with ½-inch mesh netting. Data were also collected in the area in January 1960 by the Fisheries Research Board of Canada research vessel *A. T. Cameron*, using a No. 41-5 trawl with a 1⅛-inch codend liner.

The most useful collections were made in June and October 1959 and January 1960. The length distributions for these fish are shown in Fig. 4.

To interpret the meaning of the peaks in the frequencies of the smaller fish in Fig. 4, the Petersen method of assigning ages to length–frequency modes was followed (see Graham, 1956, p. 285). With the aid of knowledge of spawning times and growth of this cod population, age I was assigned to the peak at 13 cm and age II to the peak at 19–20 cm in the June sample. The October

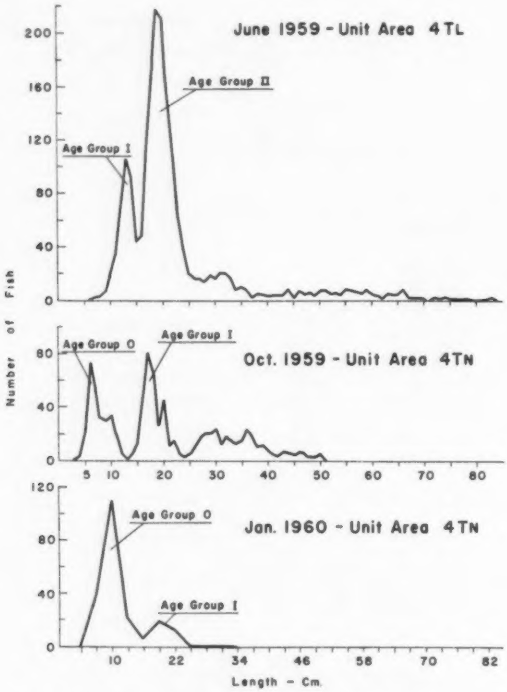

FIG. 4. Length distributions of special collections of small cod from ICNAF Division 4T taken to check age-determination methods.

sample had a peak of young-of-the-year (age-group 0) at 6 cm and the age I mode had moved to 17 cm owing to growth of the group. In the following January the age-group 0 fish were approaching their first birthday, which is arbitrarily set at February 1, and had a mode at 10 cm, while the 1-year-olds approaching their second birthday had a peak at 19 cm.

The ages of these small fish having been indicated by the length-distribution modes, the otoliths were examined. Study of their structure indicated the way in which the hyaline and opaque zones should be interpreted in order to arrive at the age of the fish. The study established that an unusually small hyaline zone appearing in the centre of some of the otoliths was an annulus. Apparently the reason for the small size of this year-zone was the relatively late summer spawning of many of these cod, which resulted in less time for growth of the otoliths of the young fish before the first annulus was laid down.

Examination of these small otoliths also showed that ageing discrepancies might arise because of the prevalence of "false checks" in some of the material. Most errors of this type have now been eliminated by use of a "ring-spacing" criterion. This criterion has been established by observations indicating that the opaque zones between each hyaline annulus normally become progressively smaller from the centre to the edge of the cross-section of the otolith. Thus, when the reader is doubtful about the validity of an annulus, the spacing of annuli in the otolith can be used as an aid in interpretation.

DIFFERENCES WITH LOCATION. Average size at age provides a means of calculating growth rates, either by comparing the differences in length or weight between a number of age-groups sampled at one particular time, or by following separate year-classes over a series of years. As is the case for many other fish species, size-at-age and growth rates vary greatly from one population to another and even from year to year within the same population.

Data for mean lengths of various age-groups of cod taken by otter trawlers in the northwest Atlantic are plotted in Fig. 5. The information concerns cod populations from four widely-separated statistical divisions laid out by the

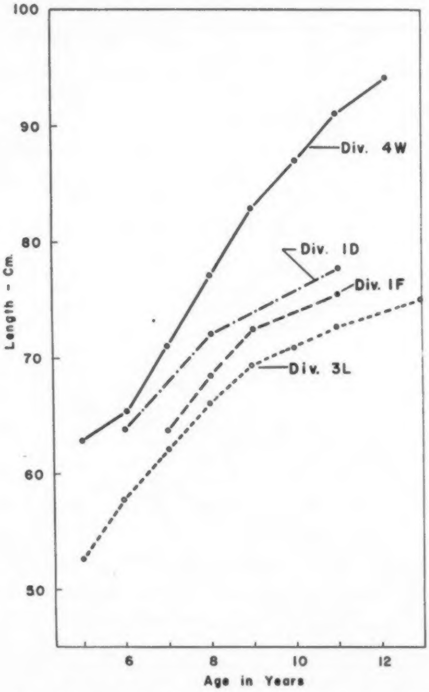

FIG. 5. Average length at successive ages of cod in the ICNAF area. Data for Division 3L for June–July, 1948–1949 are from Fleming (1960). Data for Divisions 1D and 1F for April 1958 and for Jan.–Feb. 1958, respectively, are from Meyer (1960). Data for Division 4W for Feb.–Apr., 1956–1958 are from the files of the St. Andrews Station.

International Commission for the Northwest Atlantic Fisheries (Fig. 6). Averages for Divisions 1D and 1F are from Meyer (1960). Data for Division 3L are given by Fleming (1960) in his table II. The data for 4W are from files of unpublished material at the St. Andrews Station. Variances of means for all the data were not available; however, the averages for fastest and slowest growth seemed sufficiently well spread to indicate that there must be differences in rates for different populations. The data for 5- and 6-year-olds are no doubt affected by fishermen's cull and gear selection for large fish, which would tend to bias the means upwards. Size-at-age in the data changes from area to area, being largest in Division 4W and smallest in Division 3L. The growth rates for cod from these areas, as represented by the increment in centimetres from one age-group to the next, also differ.

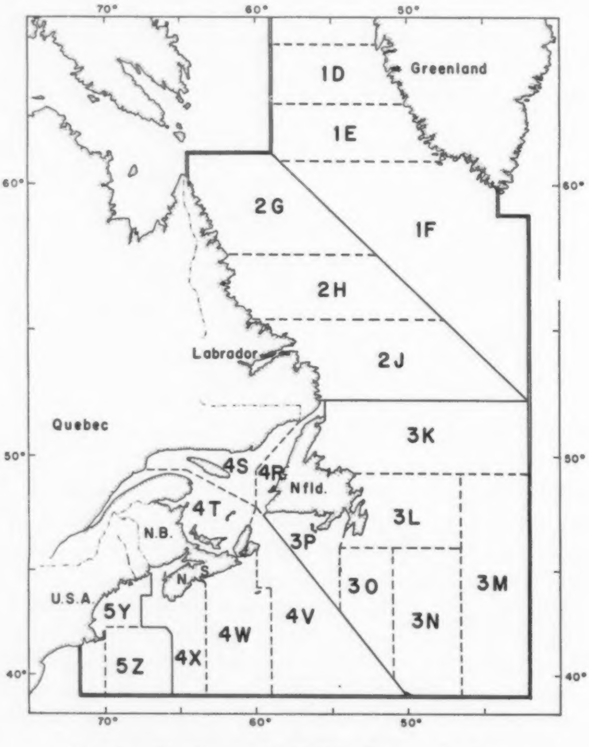

FIG. 6. Divisions of the ICNAF Convention Area.

CHANGES IN COD GROWTH IN ICNAF DIVISION 4T. A more detailed study of the growth of cod exploited in parts of Division 4T was made, using commercial age and length data. Sampling carried out during the years 1949–1958 was used except for 1954 where weighting factors for age–length data were not reliably based. The data were taken from samples of landings of a fleet of 60-ft (about 50 gross tons) draggers of similar design and power, using similar types of trawl gear and landing in the Caraquet, N.B., area. The population was studied by McCracken (1959) through a tagging investigation which showed that the concentration of cod found in the offshore northern New Brunswick area (Unit Areas 4Tk, 4Tl, 4Tn (Fig. 1)) in the "summer" (June–November) shifts to the Cape Breton area (Unit Areas 4Tf, 4Va) in the "winter" season. This shift, combined with adverse weather conditions in the winter in the northern New Brunswick area, resulted in limitation of the fishery by the Caraquet draggers in the area to the months of May to October–November. The fish have returned to the northern New Brunswick area each summer during the years they have been under observation.

Table II. Mean lengths (cm) and their standard errors for some age-groups of cod sampled from Division 4T otter-trawl landings in the May–October period, 1949–1953 and 1955–1959.

Age-group		Year of sampling									
		1949	1950	1951	1952	1953	1955	1956	1957	1958	1959
6	$\bar{x}$	57.80	56.16	58.37	58.87	58.32	60.06	60.84	62.58	58.97	55.82
	$S_{\bar{x}}$	0.75	0.74	1.09	0.99	0.75	1.35	0.62	1.84	0.87	0.56
7	$\bar{x}$	63.07	58.49	61.31	63.28	65.74	66.71	65.51	64.85	64.07	60.96
	$S_{\bar{x}}$	0.87	0.66	1.74	1.47	1.54	1.49	1.34	0.69	2.24	1.09
8	$\bar{x}$	66.57	60.57	64.41	64.61	69.03	73.90	74.00	71.36	68.08	66.33
	$S_{\bar{x}}$	0.84	1.34	0.96	1.33	2.62	1.84	1.37	2.13	1.23	2.32
9	$\bar{x}$	70.14	65.62	67.65	65.88	70.15	79.67	79.84	78.92	72.69	70.52
	$S_{\bar{x}}$	1.56	0.79	2.12	1.51	2.41	3.35	1.31	1.52	3.57	2.26
10	$\bar{x}$	76.06	67.55	72.75	70.95	73.47	77.19	82.79	82.15	78.47	74.16
	$S_{\bar{x}}$	3.02	1.66	1.94	2.08	3.48	11.10	3.77	1.79	3.90	5.38

Figure 7 shows average size of age-groups 6 to 10 over the years 1949–1959. Younger age-groups are taken in the samples but gear selection and discards of small fish at sea bias their mean lengths upwards. For age-groups over 10 years there are not usually enough fish in any one sampling year to give a good estimate of the mean length. The data used are shown in Table II.

The main change in size-at-age evident in Fig. 7 is the rise of all age-groups from a low mean length in sampling year 1950 to a high in 1955–1957 and a return to smaller sizes in 1958 and 1959. The change in growth rate in these years can be demonstrated by comparing differences in mean length of age-groups. During 1952 the difference in average length between 6- and 10-year-olds was 12.0 cm, while in 1956 it was 22.0 cm, indicating a greatly increasing increment per year in the intervening period.

In Fig. 8 the same basic data are combined and averaged to show the trends of curves during the fast- and slow-growth periods. For ages 6–10 the 1955–1957 averages for these cod are about 3–13 cm higher than those for 1950–1952. The difference in slopes of the two lines indicates a change in rate of growth between the two periods.

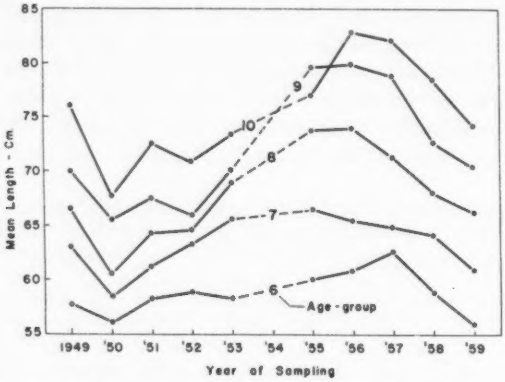

FIG. 7. Mean lengths of age-groups of cod sampled from ICNAF Division 4T otter-trawl catches landed in the area of Caraquet, N.B., during the months of May–October.

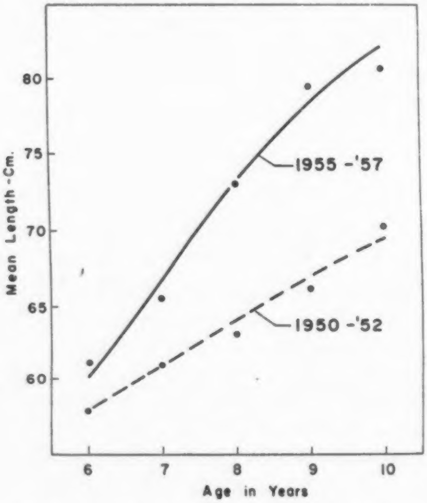

FIG. 8. Cod growth in Division 4T, May–Oct., averaged for two periods. Curves were fitted by eye to points for age-length means from commercial landings data.

Possible effects of fishing methods on results. Work by Dickie (1960) suggested disparity between mean lengths of cod in corresponding age-groups depending upon whether they were caught by line or by otter trawl. This possibility was investigated in an experiment in comparative fishing. The vessels used were the M.V. *Harengus* (84-ft otter trawler) towing a No. 36 net and the M.B. *Lameque No. 2* (55-ft longliner) fishing regular groundfish longline gear. Cod populations were fished for 3 days on the grounds in Unit Areas 4Tn and 4Tl (Fig. 1) near the New Brunswick coast. Fork lengths and otoliths were taken from all cod caught.

The percentage length composition of cod catches by the two boats (Fig. 9) showed marked differences in length selection by the two fishing gears operating on the same population of fish. The otter trawl tended to take intermediate sizes of cod (40–70 cm) as the greatest proportion by numbers of its catch. Most of the smaller fish escaped through the fairly large ($4\frac{3}{8}$ inches, stretched) meshes of the regular commercial codend used on the trawl. The line-trawl catch contained a much greater proportion of cod over 70 cm in length. Smaller cod were probably eliminated from the catch of this gear by the size of the hooks used (No. 15). The skipper of the longliner was of the opinion that most of the fish over 70 cm taken by his gear were caught as the baited gear settled to the bottom. He thought most of the larger cod were off the bottom where they would be less vulnerable to the otter trawl. This theory was supported by casual observations by the technician aboard who stated that more large fish seemed to be caught near the buoyed ends of the gear which were presumably off bottom.

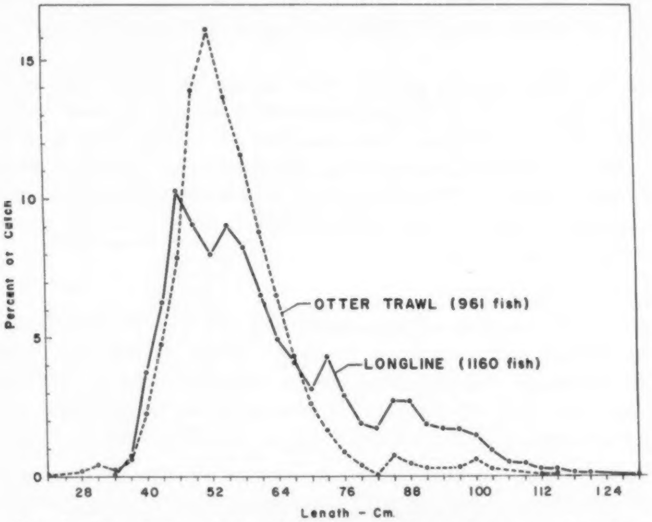

Fig. 9. Length composition of cod from longline and otter-trawl catches taken in June 1960 on the same fishing grounds.

The ages of a sample of these fish were determined to see in particular if the ones over 70 cm in the longline catch were faster-growing individuals or older ones. This was done by taking a stratified sample of otoliths, 15 per 3-cm length group where available, from those which had been collected (the theory of this type of sampling as applied to fish hage determination is reviewed by Gulland (1955)). When the otoliths were read the resulting age and length data were summarized in the form of growth curves for the two types of fishing gear (Fig. 10). There are no consistent differences in size-at-age of cod caught by the two methods. However, there was a much greater abundance of fish 8 years old and over in the longline catch than in the otter-trawl catch (Fig. 11). Thus, the cod over 70 cm in the longline catch (Fig. 9) were older fish, rather than fish which had grown faster than those caught by otter trawl.

The relative year-class strength of the younger age-groups was similar in the two samples. Hence a strong year-class (here the 1954 year-class) in this area could be detected in landings from either gear. The strong 1950 year-class shown in Fig. 3 was still noticeable in these 1960 catches of large cod by the longliner.

Thus, the differences in growth noted by Dickie (1960) did not seem to be explained by selectivity of fishing gears within age-groups over a small fishing area. This does not eliminate the possibility that there may be differences in growth rates in cod populations of the different areas normally fished by line and otter-trawl vessels. However, there is no evidence available from tagging or other studies to indicate that such a factor is involved in the growth change demonstrated in Fig. 7 and 8.

OTOLITH MEASUREMENTS

Further corroboration of the growth change in Gulf cod was sought by measuring annual increments on otoliths. Trout (1954) has demonstrated differences in yearly otolith increments in different parts of the Barents Sea stock of cod and their relation to the growth of the whole fish. His method of otolith measurement was used as a guide in the present study.

METHODS. The objective was to investigate whether an increase in growth of the otoliths of cod coincided with the apparent increase in growth in length shown in Fig. 7. For this purpose otoliths used were broken in the usual way for age reading. The surface of the posterior piece of the left-hand otolith was then ground down until the fracture surface was smooth and in the same relative position on each one. It was placed in plasticine and the fracture surface covered with glycerin solution in the normal way.

The instrument used for measuring distances between rings was a stereoscopic microscope, manufactured by Watson of England, and fitted with a special screw micrometer eyepiece. The otolith was viewed using 6× oculars, 1.25× objectives and reflected blue light. The drum on the screw micrometer was divided into 100 divisions for one complete rotation and one of these divisions spanned 0.0048 mm of the field of view. Experimentation showed a maximum of two to three divisions variation when measuring the same sequence of annuli a

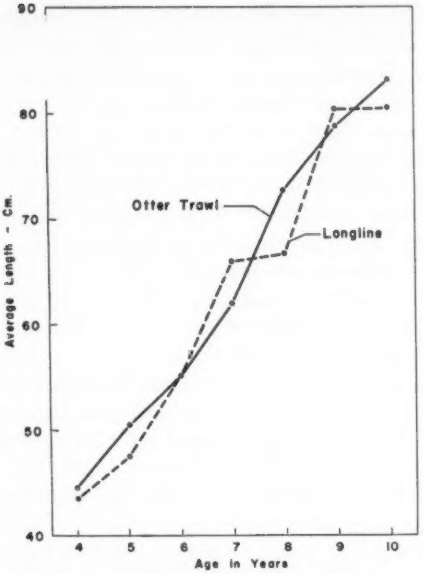

FIG. 10. Growth curves of cod from longline and otter-trawl catches taken in June 1960 on the same fishing grounds.

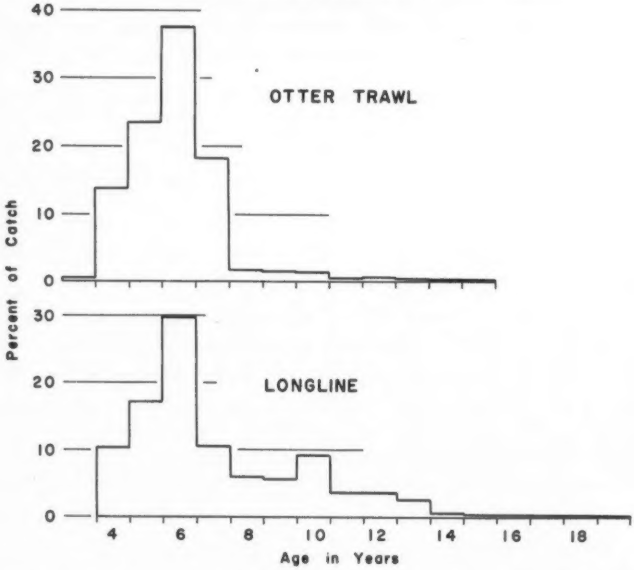

FIG. 11. Age composition of cod from longline and otter-trawl catches taken in June 1960 on the same fishing grounds.

number of times. Thus, when the inner edges of hyaline annuli to which measurements were made were clearly defined, the instrument gave acceptable measurements. All measurements were recorded in terms of one unit on the rotating drum, and called one eyepiece unit (e.p.u.).

Measurements were made from the inner edge of one hyaline ring to the inner edge of the next hyaline ring, except in the case of the first measurement which was from the centre or origin in the initial opaque growth to the inner edge of the first hyaline year ring. This grouping was adopted so that each measured increment would correspond approximately to a calendar year of life for these cod, where hyaline growth is found in the winter and spring, and opaque growth in the summer and early autumn. The axis of measurement was from the origin to the edge at the middle of the concave side of the cross-section, the side opposite the one having the *sulcus acusticus*.

Increments on these cod otoliths are irregular during their first 2 or 3 years of life so selections were made to eliminate use of these increments in comparisons and to give information on variation caused only by differences in growth conditions during the years in question. Trout's (1954) fig. 2 shows a definite decrease in otolith increment with age. This also appeared in the data dealt with here. Thus, to demonstrate growth increments differing in two different years, say 1954 and 1952, it was necessary to compare growth of cod otoliths at the same age in those two years. For instance, the distance from age ring 5 to age ring 6 was compared on fish of the same age, sampled in the two different

TABLE III. Measurements of yearly increments on 10-year-old Division 4T cod otolith cross-sections, sampled in 3rd quarter, 1957; measurements in eyepiece units (defined in text).

Code no.	Centre to ring 1	R1–R2	R2–R3	R3–R4	R4–R5	R5–R6	R6–R7	R7–R8	R8–R9	R9–R10
X4186	39	68	96	55	45	36	38	29	26	18
X4188	44	78	64	47	39	27	26	25	32	22
X4194	76	70	60	41	41	33	21	23	19	14
X4288	63	106	57	51	34	25	22	28	19	23
X4324	24	65	46	46	39	34	32	45	40	33
X4316	54	66	51	58	52	33	33	38	34	33
X4315	46	53	96	42	42	39	48	41	26	30
X4313	62	67	78	58	45	35	34	36	33	23
X4311	23	68	55	51	47	44	35	48	49	37
X4864	67	42	86	36	41	30	41	27	28	26
X4853	81	68	33	35	39	48	43	45	37	28
Mean ($\bar{x}$)	52.6	68.3	65.6	47.3	42.2	34.9	33.9	35.0	31.2	26.1
$S_{\bar{x}}$	5.8	4.7	6.2	2.5	1.5	2.0	2.6	2.7	2.7	2.1

years. For this sort of comparison the most suitable data available were measurements on 10-year-old fish sampled in 1957 and 1959. This allowed comparison of the fast-growth period 1953–1955 against slower-growth periods in 1950–1952 and 1956–1958. The summary of data used is shown in Tables III and IV.

TABLE IV. Measurements of yearly increments on 10-year-old Division 4T cod otolith cross-sections, sampled in 3rd quarter, 1959; measurements in eyepiece units.

Code no.	Centre to ring 1	R1–R2	R2–R3	R3–R4	R4–R5	R5–R6	R6–R7	R7–R8	R8–R9	R9–R10
T2462	42	55	44	62	39	41	33	35	23	21
T2453	43	45	51	74	51	43	38	31	22	17
T2926	70	66	48	58	50	35	24	20	20	21
T2752	37	38	60	49	50	50	35	31	28	24
T2930	41	65	45	51	42	49	48	39	44	34
T2732	34	50	64	43	61	50	37	22	23	21
T2654	45	50	43	51	48	34	30	25	21	21
T2473	51	65	77	42	42	27	28	19	17	17
T2206	76	51	45	28	31	34	28	26	20	21
T2344	43	80	92	67	44	43	41	34	31	20
T2555	26	78	52	30	40	25	23	25	19	19
T2553	43	57	37	52	46	47	44	30	15	26
T2566	38	56	61	32	38	33	31	26	23	23
T2576	41	54	48	41	50	41	28	29	23	23
T2582	61	76	62	50	69	65	56	45	33	30
Mean ($\bar{x}$)	46.1	59.7	55.3	48.7	46.7	41.1	34.9	29.1	24.1	22.5
$S_{\bar{x}}$	3.5	3.2	3.8	3.4	2.4	2.7	2.5	1.8	1.9	1.2

RESULTS. The average increments for the two sampling years are shown in Fig. 12. It is of interest to note in this figure the rise in increment in the second year. This was the result of a small increment in the first year, caused by late spawning of these fish and a resultant small amount of opaque growth included in the measurement. This first increment measured represents only about 5–7 months of growth as compared to a full year's growth measured in the outer part. The trend toward smaller increments, the older the fish becomes, is apparent in both samples.

Differences in average otolith growth are evident in the 1957 and 1959 samples. For the 1957 sample the figure shows that the curve for otolith growth was depressed in 1951 and 1952 and rose in 1953 and 1954. The increments for

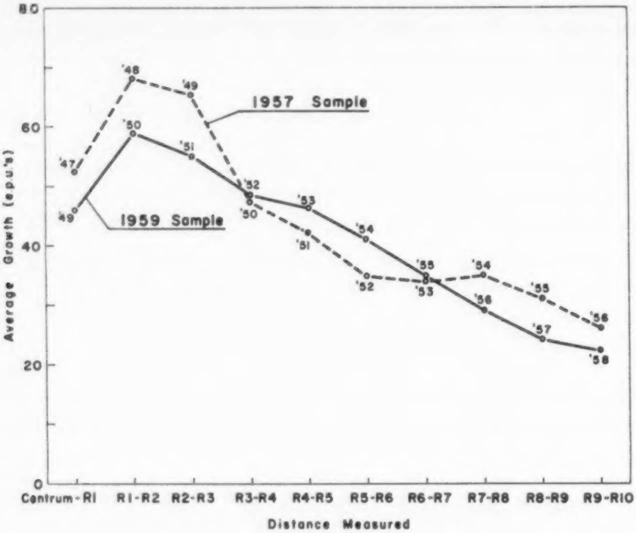

FIG. 12. Yearly increment on the periphery of otolith cross-sections from 10-year-old cod sampled from ICNAF Division 4T.

1954 and 1955 averaged considerably higher than for the years 1956 and 1957 in the 1959 sample, the years where the increase in fish growth stopped. The 1959 sample gave higher increments for 1953 and 1954 growth than that for corresponding ages in 1951 and 1952 in the 1957 sample. The latter were slow-growth years (Fig. 7). Thus, the measurements of otoliths support other evidence of a growth change in the fish.

Because of lack of age–length data from Division 4T for 1954 it has been difficult to pin down the exact years when growth rate increased. Assuming that the otolith growth reflects the growth of the fish in any one year, some use can be made of these measurements to show more specifically which years were the more important in changing the size-at-age of Gulf cod. To carry this out a method for showing "goodness of growth" in various years by comparing growth rings on scallops (*Pecten grandis* Solander) given by Dickie (1951, pp. 11–13) was followed.

An upper asymptote for average otolith measurements (Table V) was found by plotting W_r, W_{r+1} points for otolith rings 3 to 10 as shown in Fig. 13, where

W_r = average increment from the centrum to any annulus (in e.p.u.'s), and

W_{r+1} = average increment from the centrum to the annulus outside "r".

Following the method of Walford (1946) the asymptote is read off the graph (Fig. 13) where the straight line fitted to the data by eye passes through the point where $W_r = W_{r+1}$. In this case the value so found is 648 e.p.u.'s (K).

Average data on increments to successive rings (Y) were then used in calculations of K–Y (Table V) and log (K–Y). Values of log (K–Y) were plotted

TABLE V. Average increments (in eyepiece units) on 10-year-old Division 4T cod otoliths and their relation to asymptotic length (see text).

Sampling year	From centre to:									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
1959	46.1	105.2	160.5	209.2	255.9	297.0	331.9	361.0	385.1	407.6
1957	52.6	120.9	186.5	233.8	276.0	310.9	344.8	379.8	411.0	437.1
						used to estimate K				
Av. (Y)	49.4	113.1	173.5	221.5	266.0	304.0	338.3	370.4	398.1	422.3
Av. K–Y			474.5	426.5	382.0	344.0	309.7	277.6	249.9	225.7
log (K–Y)			2.676236	2.629919	2.582063	2.536558	2.490941	2.443419	2.397766	2.353532
			Age 2 + WO[a]	3 + WO	4 + WO	5 + WO	6 + WO	7 + WO	8 + WO	9 + WO

K = asymptote 648.0 e.p.u.'s.

[a] + WO = summer growth complete (wide opaque).

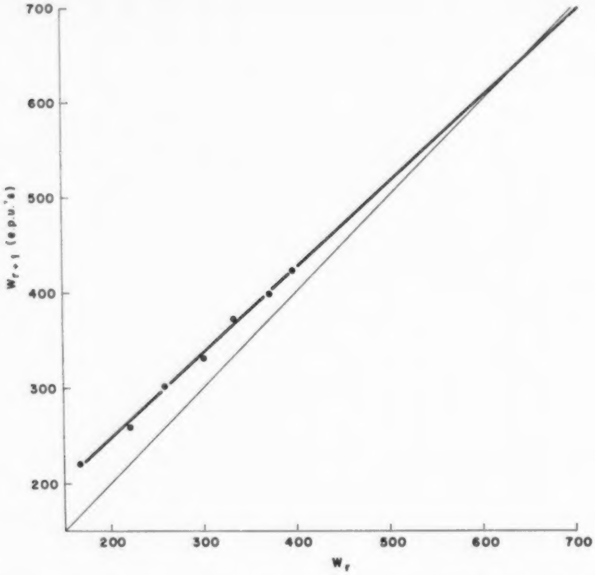

FIG. 13. Graph used to estimate asymptote (K) of accumulated average annual increments on 10-year-old Division 4T cod otoliths.

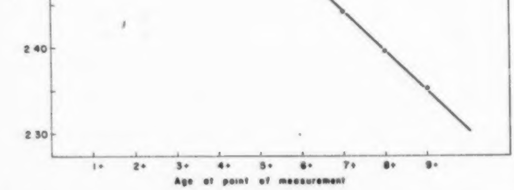

FIG. 14. The relation between measurements on otoliths and age at that otolith size. K is the estimated maximum average measurement of the otolith and Y is the average size of the otolith attained at each age. K-Y is the size still to be attained at any given age, if the fish were to live for an indefinite number of years.

TABLE VI. Average growth to be achieved and its logarithm on measured otoliths from Division 4T cod; theoretical maximum size of otolith 648 e.p.u.'s (K); actual size is Y (sampling years 1957 and 1959).

		Growth year																			
		1949		1950		1951		1952		1953		1954		1955		1956		1957		1958	
Age	Measurement	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)	K–Y	log (K–Y)
years																					
9+	Centre to R10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	210.9	2.324	...	...	240.4	2.381
8+	R 9	...	...	...	...	...	...	...	...	...	...	...	...	237.0	2.375	...	...	262.9	2.420	...	...
7+	R 8	...	...	...	...	...	...	...	...	...	...	268.2	2.428	...	...	287.0	2.458	...	...	...	...
6+	R 7	...	...	...	...	...	...	...	...	303.2	2.482	...	...	316.1	2.500	...	...	...	...	...	...
5+	R 6	...	...	...	...	...	...	337.1	2.528	...	...	351.0	2.545	...	...	...	...	...	...	...	...
4+	R 5	...	...	...	...	372.0	2.570	...	...	392.1	2.593	...	...	...	...	...	...	...	...	...	...
3+	R 4	...	...	414.2	2.617	...	...	438.8	2.642	...	...	...	...	...	...	...	...	...	...	...	...
2+	R 3	461.5	2.664	...	...	487.5	2.688	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Per cent of average annual growth achieved by year-classes and age-groups measured

	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958
9+	...	...	...	...	...	...	...	110	...	85
8+	...	...	...	...	...	...	105	...	80	...
7+	...	...	...	...	...	125	...	70	...	...
6+	...	...	...	...	99	...	120	...	...	...
5+	...	...	...	100	...	102	...	...	...	...
4+	...	...	100	...	105	...	...	...	...	...
3+	...	102	...	100	...	...	...	...	...	...
2+	...	...	...	...	...	...	...	...	...	...
Average:		102	100	100	102	113	113	90	80	85

against age and a straight-line fit to these points could be made by eye (Fig. 14). The data on increments for successive years given in Table V were then entered as individual years as K–Y and log (K–Y) in Table VI so that a comparison could be made between them and the average otolith growth data shown in Fig. 14.

For example we take data for the increment on a 2+ year otolith (Table VI) in 1949 (log (K–Y) = 2.664) and plot this on the average line in Fig. 14, then take the log (K–Y) value for the same (average) otolith at 3 + (log (K–Y) = 2.617). When this is plotted, growth taking place mostly in 1950 is represented between the two points. The percentage of an average full year's growth achieved for this particular measurement is indicated (by reading off the abscissa). This has been entered for averages for sampling years 1957 and 1959 in the bottom of Table VI. The values are arranged so that a grand average for growth deviations in the years 1949–1958 can be calculated and this is included in Table VI and plotted in Fig. 15.

Figure 15 indicates that the otolith increment for the 1954 sampling year, which is missing from the age-length sampling (Fig. 7), showed a positive deviation from average growth. The figure also shows that, of the 3 good years 1953, 1954 and 1955, the latter 2 were the best, and that there seems to have been a recession in growth in 1956, 1957 and 1958.

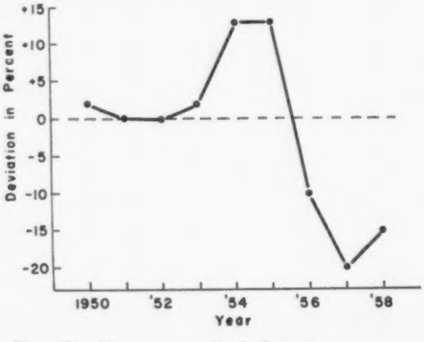

Fig. 15. Percentage deviation from average annual increment on Division 4T cod otoliths.

Growth from Tagging Data

Evidence from tagging experiments confirming the growth change in Division 4T cod is contained in a paper by Kohler (1962). Here, as stated in the summary, "an analysis of growth data from cod taggings in the Gulf of St. Lawrence in 1955 and 1956 was carried out to determine the usefulness of such data in cod growth studies. Four sets of data were examined, two for fish recaptured after one year and two for fish recaptured after two years. "Walford" transformations fitted to the tagging growth data and to corresponding commercial age–length data showed good agreement." Specifically, the 1955–1956 growth of tagged cod was shown to be greater than the 1956–1957 growth. Also for 2-year periods of growth of tagged fish, 1955–1957 was shown to be greater than 1956–1958. This independent check on growth changes agreed well with conclusions reached from examination of age–length data (Fig. 7 and Table II).

GROWTH EXPERIMENTS

Preliminary results of parts of this experiment have been published in a general form at an earlier date (Kohler, 1959). The results are dealt with here in more detail.

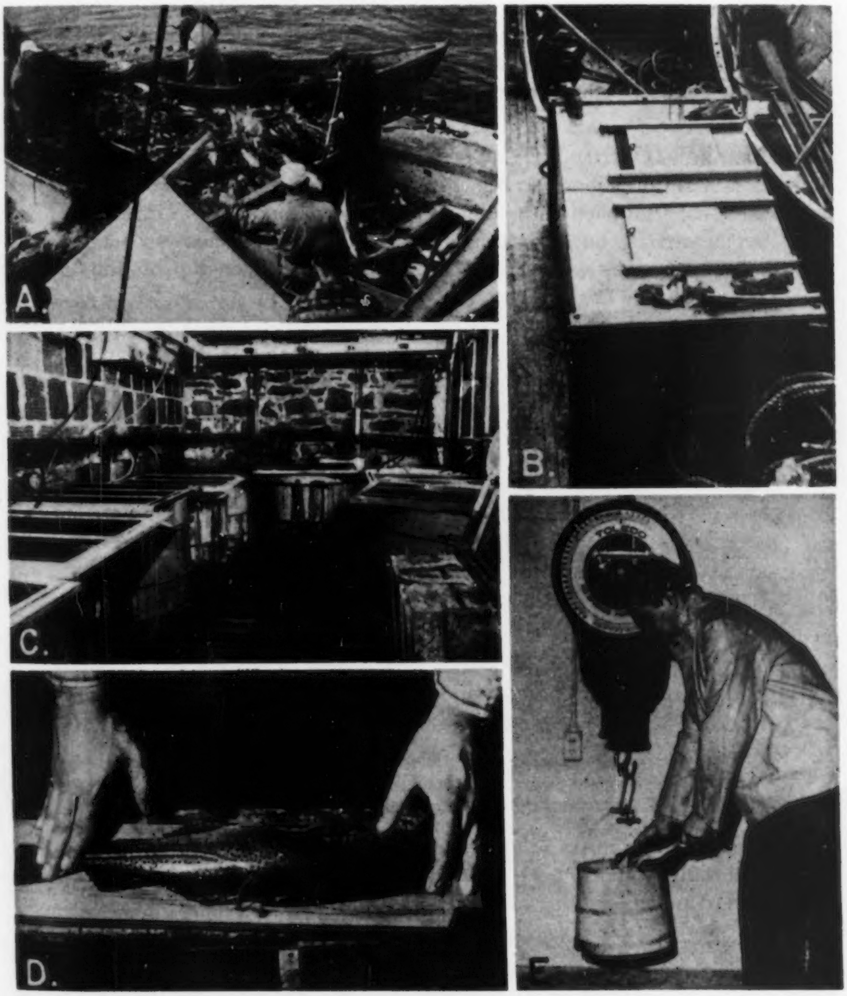

Fig. 16. A. Live cod and pollock being taken from Lockeport, N.S. trap; B. Transportation tank aboard M. B. *Mallotus*; C. Experimental tank room at St. Andrews Station; D. Length measurement of live cod; E. Weighing of live cod.

METHODS

COLLECTION AND TRANSPORTATION OF COD. The fish used for experimental work had to be in good physical condition. In order to obtain cod that had no injuries due to capture, it was decided to take them from the commercial shore traps near Lockeport, N.S. Figure 16A is a picture of the particular seining of the trap from which the experimental fish were obtained on July 8, 1957. The cod were mixed with many pollock which were taken in the same haul. The cod were dipped out with scoop nets and handed aboard the Fisheries Research Board of Canada vessel M.B. *Mallotus* where they were placed in the enclosed tank shown in Fig. 16B. A pump shown in the upper left corner of the photo circulated fresh sea water through the tank during the trip to St. Andrews.

HOLDING FACILITIES. On arrival at St. Andrews the fish were placed in the puncheon-type tanks shown in Fig. 16C (90 cm diameter by 64 cm deep). These tanks were each supplied with running sea water, pumped from Passamaquoddy Bay and aerated through air-stones attached by rubber hose to a large compressor. The screens seen in the picture were placed on top of the tanks to foil attempts by the fish to jump out.

Six of these tanks were used for the experiment. As a preliminary step the captured cod were divided into 2 size-groups, one of 33–40 cm and the other 40–46 cm. Then each of these 2 groups was divided into 3 equal parts and the resulting 6 groups were placed in separate tanks. Numbers of fish in each of the tanks at the beginning and end of the experiment are shown in Table VII.

TABLE VII. Numbers of cod in tanks at beginning and end of the experimental period.

Tank no.	Initial size of fish	Rate of feeding	Number of cod	
			Beginning	End
5	large	maximum	7	2
6	small	maximum	17	8
7	large	intermediate	8	3
4	small	intermediate	17	4
9	large	maintenance	6	3
8	small	maintenance	17	7

The reduction in numbers in each tank as the experiment progressed is due in part to mortalities and in part to removals of fish in poor shape. Any cod that was observed to be behaving unusually or whose physical condition deteriorated badly in a 3-week period was removed from the tank at the end of that period.

PRELIMINARY TRIALS. From July 10 until August 22 trial-and-error experiments were carried out to discover the best methods of feeding in order to achieve a maximum, intermediate and maintenance feeding rate in one of each of the tanks containing large and small cod. The maximum rate of feeding for large and small fish was that rate at which the fish would consume the most food over a period of a year. The maintenance rate of feeding for both sizes was that rate at which the average weight of fish in the tank could be kept constant over the experimental period. The intermediate rate was between these two for both large and small fish; for large fish it was arbitrarily set at 82% and for small fish 71% of the maximum rate (Table VIII). Herring was selected as a natural food which was easily available, kept well frozen, and most important of all, seemed to be the food involved in the growth change being investigated in ICNAF Division 4T. The trials showed that, if the cod were fed a limited amount of food each day, the amount dictated by the feeding rate desired, the more aggressive fish in the tank got most of the food while some got virtually none at all. It was found that, to establish the three rates, the fish in each tank had to be given all they would take at one feeding, the differences in rates being established by the number of times they were fed in any one 3-week period.

TABLE VIII. Average yearly food consumption of cod, and conversion of food (frozen whole herring) to cod weight.

Rate of feeding		Initial size	Food consumed	Food for maintenance	Food for growth	Average growth	Conversion factor
		g	g	g	g	g	
Maximum	(Large)	757	3025	1375	1650	743	2.2
Maximum	(Small)	445	2736	1176	1560	697	2.2
Intermediate	(Large)	748	2475	1375	1100	454	2.4
Intermediate	(Small)	448	1956	1176	780	377	2.1

ROUTINE PROCEDURES. Cod on maximum rations were usually fed five times in a 3-week period; those on intermediate rations three times; those on maintenance rations twice, after the trial period was concluded. The excess herring were removed 24 hr after the feedings and their weight, corrected by a factor for water absorption, was subtracted from weight of herring introduced the previous day to give the amount consumed.

The experiment proper was started on August 22, 1957, and lasted until September 4, 1958, giving 54 weeks of continuous records. Just prior to the start some fish in each tank were tagged in order to have records of growth of individuals as well as averages for the six groups. Subsequently it was found that there were no consistent differences between the growth of the tagged and untagged individuals (Kohler, 1962).

Measurements of growth in length and weight were taken for each individual every 3 weeks. Figures 16D and 16E demonstrate the techniques used. Length was measured to the nearest millimetre on a standard fish-measuring board with headpiece. Weights were recorded to the nearest half ounce using the Toledo scale shown in Fig. 16E and placing the fish in a buttertub full of water during the operation. At this time notations as to the condition of the fish were made, including spawning state, wounds, infections, condition of tag scar.

Temperature of the water in the tanks was recorded each day. Since the water was pumped direct from Passamaquoddy Bay to a small reservoir and thence to the tanks, the water temperature fluctuated in the same fashion as the Bay water, with a late summer high and a winter low.

In order to ensure that the fish were in approximately the same condition, not varying greatly in relative plumpness, their initial lengths and weights were plotted in Fig. 17. The double log plot of data for both initially small and initially large fish indicates a straight-line relationship with no extremely thin or fat fish evident.

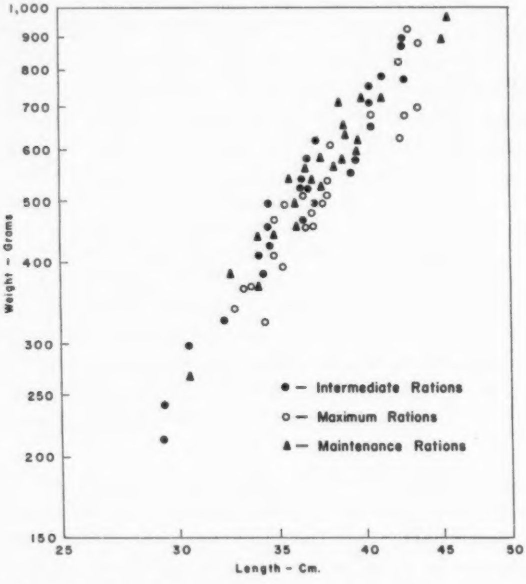

FIG. 17. Length–weight relationship for cod at the beginning of the growth experiment.

FEEDING AND GROWTH

MAINTENANCE-FED FISH. In order to separate amounts of food required to maintain the fish at a constant weight from that used for growth, one tank of the large and one tank of the smaller fish were kept on maintenance rations. The amount fed to these fish to keep them at a constant size had to be established on a trial-and-error basis, weighing them every 3 weeks to see whether a gain or loss had been registered. Over the year the approximate maintenance feeding schedule was defined and, at the end, a 45-week period was selected for initially large fish and a 33-week period for initially small fish, at the beginning and end of which the average weight of cod in the tank was the same. For use with other growth data which have been summarized on a yearly basis, the maintenance requirements (Table VIII) to cover a 52-week period were calculated. The data showed that for the initially large cod (average weight 751 g) 1375 g of frozen herring per cod were required to maintain constant average weight for a year. The tank of initially smaller cod (average weight 513 g) required 1176 g per fish per year for maintenance.

FISH FED AT INTERMEDIATE AND MAXIMUM RATES. One tank of large cod and one of small cod were each fed at the previously defined intermediate rate. The two remaining tanks, one of large and one of small fish, were kept on maximum rations. Average food consumption and average growth in grams are shown for each of these four tanks in Fig. 18 and Table IX. Growth data for males and females are not separated because information from cod surveys (Jean, 1960, personal communication) in ICNAF Subarea 4 has shown no

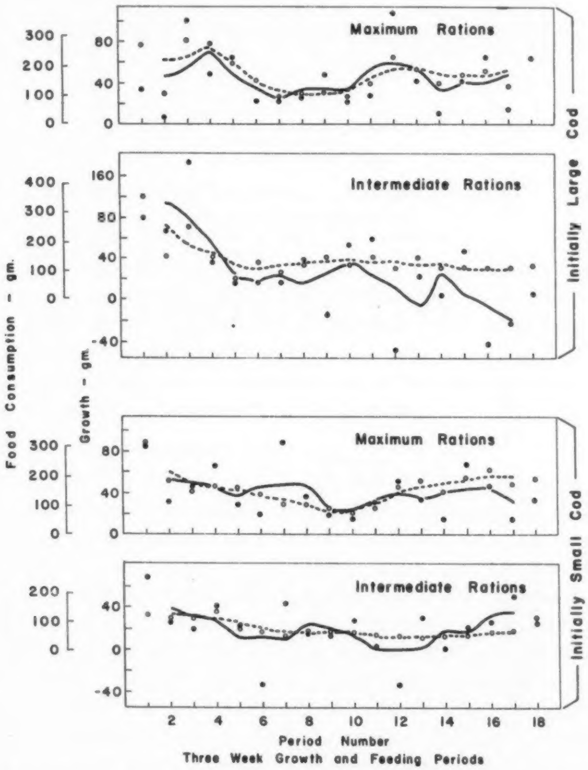

FIG. 18. Average growth of cod in 4 separate tanks from Aug. 22, 1957, to Sept. 4, 1958. Growth (solid line) and food consumption (broken line) were summarized for 3-week intervals (2 exceptions). The curves are from the data smoothed by 3's.

TABLE IX. Average temperature, growth and food consumption of cod by 3-week periods in experimental tanks.

3-week growth period	Av. water temp.	Small cod Maximum rations				Large cod Maximum rations				Small cod Intermediate rations				Large cod Intermediate rations			
		No. fish	Food consumed	Growth[a]		No. fish	Food consumed	Growth		No. fish	Food consumed	Growth		No. fish	Food consumed	Growth	
	°C		g	oz	g		g	oz	g		g	oz	g		g	oz	g
1	13.6	17	304	3.0	85	7	268	1.2	34	16	256	2.5	71	7	355	2.8	79
2	13.3	16	181	1.1	31	6	102	0.2	6	16	103	1.0	28	5	149	2.4	68
3	11.3	15	155	1.6	45	5	289	3.5	99	14	126	0.7	20	4	256	4.7	133
4	10.1	15	166	2.3	65	6	276	1.7	48	14	126	1.5	42	4	147	1.3	37
5	8.0	14	161	1.0	28	6	212	2.2	62	14	116	0.8	23	4	77	0.6	17
6	6.5	14	136	...[b]	..	5	156	...[b]	..	12	84	...[b]	..	4	129	-1.6	-45
7	4.5	13	107	...[b]	..	5	99	...[b]	..	12	73	...[b]	..	4	99	2.8	79
8	3.7	11	106	1.3	37	3	101	1.0	28	11	61	0.6	17	3	124	1.3	37
9	2.3	11	84	0.7	20	3	115	1.7	48	11	60	0.5	14	3	147	-0.5	-14
10	3.8	9	76	0.5	14	3	89	0.8	23	10	67	1.0	28	3	121	1.9	54
11	4.2	9	90	1.1	31	3	143	1.0	28	8	63	0.1	3	3	147	2.1	60
12	6.0	9	164	1.8	51	3	232	3.8	108	8	95	...[b]	..	3	113	-1.8	-51
13	7.4	8	181	1.2	34	3	189	1.5	42	8	94	1.1	31	3	141	0.8	23
14	9.6	8	141	0.5	14	3	145	0.4	11	8	94	0.1	3	3	110	0.2	6
15	10.5	8	199	2.4	68	3	173	1.5	42	7	108	0.8	23	3	112	1.7	48
16	12.2	8	225	1.6	45	3	181	2.3	65	4	157	1.0	28	3	111	-1.5	-43
17	13.2	8	175	0.5	14	2	136	0.5	14	4	192	1.9	54	3	110	-0.8	-23
18	13.4	8	191	1.2	34	2	236	2.3	65	4	156	1.0	28	3	121	0.2	6

[a] Due to equipment available, growth of individuals was measured in ounces to nearest half ounce, then converted to grams.

[b] Suspected inaccuracy in weighing.

consistent difference in average rates between sexes. The data are shown by 3-week periods for a total of 54 weeks, the duration of the experiment. Since the basic day-to-day data on food consumption and growth of individuals are so voluminous, they are not included in tabular form but are on file at the Biological Station, St. Andrews, N.B.

The plot of food consumption and growth in Fig. 18 shows certain trends. Food consumption for both large and small cod on maximum rations fluctuates from a summer high to a winter low, reaching the low point in the January–February period. This is coincident with the rise and fall of water temperature in the tanks (Fig. 19 and Table IX) and is dealt with further in a later section. Food consumption of fish on intermediate rations was initially high then levelled off at a lower rate due to limitation of the amount of herring made available in any 3-week period.

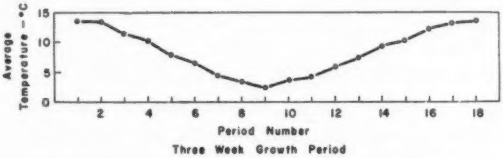

FIG. 19. Average water temperatures in the experimental tanks for each 3-week period.

Growth of cod on maximum rations generally dropped to a low coincident with the low in winter feeding, but some positive growth was registered at all times of the year. Fish on intermediate rations grew more irregularly from period to period and the trend throughout the year is not well defined. The initially smaller ones showed a tendency to follow the pattern of those on maximum rations, with faster growth in the summer.

LENGTH AND WEIGHT INCREMENT FOR YEAR

Totals of growth in length and weight for 3-week periods gave average growth of fish of different initial sizes and on different feeding rates for 54 weeks. These growth totals were reduced by 52/54 to give averages for one year (Table X). The table shows that large and small fish on maximum rations grew more in both length and weight than their counterparts on intermediate rations. However, the differences in length increment for the two rates of feeding were much more noticeable for initially small cod than for the larger ones. Differences in weight increments are not quite so obvious until expressed on a percentage increase basis. Here, Table X shows that initially large fish almost doubled their weight on maximum rations while increasing by little more than half on intermediate rations. Initially smaller fish increased more than 1.5 times in weight on maximum rations while those on intermediate rations gained close to $\frac{7}{8}$ of their initial weight. The extent to which change in fish weight is dependent on water content in the flesh has been dealt with by Love (1960). In freshly-caught cod, water content decreases from 83.5% in March to 79.5%

in October and can increase to 88% among cod starved to the point of death. It seems unlikely that water content among these feeding fish will seriously distort calculations on food conversion.

TABLE X. Average growth in length and weight of cod during 52 weeks in captivity.

Rate of feeding	Initial			Growth in		Increase in weight
	Size	Length	Weight	Length	Weight	
		cm	*g*	*cm*	*g*	%
Maximum	Large	42.5	757	10.9	743	98
Maximum	Small	34.4	445	14.9	697	157
Intermediate	Large	41.2	748	10.2	454	61
Intermediate	Small	35.6	448	8.9	377	84

FOOD CONSUMPTION AND CONVERSION TO COD WEIGHT

Figures on food consumption for 52 weeks were calculated in the same way as for growth in order to examine consumption and conversion of frozen herring to weight of live cod. Table VIII shows that average yearly food consumption per fish in the tanks was greater at the maximum rate of feeding for both large and small fish than it was at the intermediate rate. In order to calculate amounts of food used for growth by the cod in each tank, the amount of food used for maintenance (determined as described on p. 82) was subtracted from the total food consumption for one year. To find the efficiency of conversion of herring to cod weight, total average growth in weight for the year was divided into the amounts of food used for growth to get the conversion factor (Table VIII).

The final figures for these conversion rates for a year of feeding were quite similar. Maximum-fed fish of either initial size converted at an average rate of 2.2 g of herring for growth producing 1 g of cod weight. The conversion rate for those on intermediate rations varied from 2.4 for initially large fish to 2.1 for the smaller fish. Comparisons of this rate of conversion with those published for other fish are complicated by the fact that most publications do not have data on maintenance requirements separated from food used for growth (see Brown, 1957, p. 386). However, Dawes (1930, 1931) shows that, in his experiments in controlled feeding of *Mytilus* to plaice, fish on maximum rations converted at a rate of 4–5 g of food for growth to 1 g in increase in weight of fish. The efficiency of conversion here is not as great as that shown by our cod. Bückmann's (1952) experiments on the plaice gave what seem to be better conversions although only overall figures, including maintenance rations, are mentioned. He indicated that his "food quotient" of 5.8 g food to 1 g plaice showed considerably greater efficiency than data he refers to from Dawes (1930, 1931), giving a 10.8:1 ratio. For freshwater fish, Brown's (1957) summary shows that on live food alone conversion factors from 2.3 to 7.1 have been found for brown trout (as just mentioned, these factors include maintenance requirements).

Indications here are that some of the factors responsible for variation, aside from intrinsic calorific value of the food itself, are size of the fish, appetite, water temperature, spawning, and cycles in growth related to yearly seasons.

SPAWNING ACTIVITY IN THE TANKS

When measuring and weighing the experimental fish on March 20, 1958, it was first noticed that some of them were in a ripe stage of maturity. Eggs or milt from certain individuals were expelled when they were being handled. Records of numbers of fish in the tanks in this condition were kept from this time until the end of the experiment. The ratio of fish in spawning condition during each measuring period is shown in Table XI.

TABLE XI. Ratio of fish recorded in a ripe state of maturity in tanks during growth experiments, 1958.

Tank no.	March 20	April 10	May 1	May 22	June 12	July 3	July 24	Aug. 14	Sept. 4
4	$\frac{3}{10}$	$\frac{2}{8}$	$\frac{1}{7}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{7}$	$\frac{2}{4}$	$\frac{2}{4}$	$\frac{1}{4}$
5	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{2}$	$\frac{0}{2}$
6	$\frac{2}{9}$	$\frac{3}{9}$	$\frac{2}{9}$	$\frac{2}{8}$	$\frac{2}{8}$	$\frac{2}{8}$	$\frac{2}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
7	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{1}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$
8	$\frac{0}{9}$	$\frac{1}{9}$	$\frac{0}{9}$	$\frac{0}{9}$	$\frac{1}{9}$	$\frac{0}{9}$	$\frac{0}{9}$	$\frac{0}{8}$	$\frac{0}{7}$
9	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{1}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$	$\frac{0}{3}$

The expulsion of spawning products in the tanks undoubtedly affected the average weight of cod from period to period but there was no way of correcting for this. Since not all individuals spawned and not all spawners reached the running stage at the same time, changes due to this phenomenon were not evident at the periodic measuring times.

In tanks 8 and 9 where the cod were on maintenance rations, very little spawning activity took place, there being only three observations of one spawner each on April 10, May 22 and June 12, 1958. This may indicate that when food supplies are low cod fail to complete the maturing process, especially at sizes where they are likely to be maturing for the first time. A comparison of average initial lengths (Table X) with length at first maturity of cod in fig. 2 of Powles (1958) shows that this was probably their first time for spawning. However, in tank 5 where three initially large fish were on maximum rations no spawning was observed.

In tanks 4 and 6, where most of the ripe fish were found, the condition persisted over most of the summer. This was during a water temperature rise from about 4° to 13°C (Fig. 19). There were changes in appetite during this period also, as will be seen in a later section on water temperature–food consumption relations. The rather irregular changes in stage of maturity did not seem to bear any constant relationship to either of these factors. Thus, while there undoubtedly were some variations in weight due to spawning, it was assumed that these did not seriously affect data on other relationships that came out of these experiments.

TEMPERATURE, FEEDING, AND GROWTH RELATIONS

Averages of food consumption and growth exhibited a downward trend as the winter season approached, with an increase in the spring (Fig. 18). The only obvious environmental factor that changed in a like manner in the tanks was temperature (Fig. 19). The amount of food that was offered to fish on maximum rations remained at a level higher than they would consume, so the variation in food consumption was due to changes in appetite rather than lack of food to eat.

In order to examine the relationships involved between temperature, food consumption, and growth, partial correlation coefficients were calculated using the data shown in Fig. 18 and 19 for 3-week periods. The method of calculation followed was that given by Snedecor (1946, pp. 357–9) and the results are shown in Table XII.

The column in Table XII giving the relationship between food consumption of cod and water temperature in the tanks shows a good positive correlation for initially small cod on both maximum and intermediate rations, and for initially large cod on maximum rations. A rise in food consumption was associated with a rise in temperature. Since the fish were always offered more than they would take at any one feeding, variations in feeding at all but the extreme ranges of temperature, at least, can be considered to be direct reflections of appetite. However, it is probable that in the summer the temperature in the tanks (13–14°C) was reaching the upper critical limit for feeding for large cod (see McKenzie (1935) for earlier evidence of this effect). Brown (1957) has reviewed the problem of varying appetite and points out that this phenomenon seems to be correlated with environment, temperature and crowding being two of the most pertinent factors. The crowding factor did not vary greatly within either size-group of fish held in most of the 3-week periods as the experiment progressed (Table IX).

The coefficient for the temperature–food consumption relation for large cod on intermediate rations indicated poor correlation. This effect was the result of experimentally limiting one variable, food consumption, to the extent that it was not allowed to fluctuate normally with changing temperatures as in the other tanks.

The correlations between growth and food consumption for the same tanks of cod are also shown in Table XII. The "*r*" values for these data again indicate a significant positive correlation in each tank, except for the one containing large cod on intermediate rations. It might have been better to have been able to

TABLE XII. Partial correlations of growth (g), temperature (t), and feeding (f), for the cod growth experiment.

Conditions in tank	Coefficient of correlation between (t) and (f) independent of (g) ($r_{tf \cdot g}$)	Degrees of freedom	Significance (Snedecor, 1946, p. 351)	Coefficient of correlation between (f) and (g) independent of (t) ($r_{fg \cdot t}$)	Degrees of freedom	Significance (Snedecor, 1946, p. 351)	Coefficient of correlation between (t) and (g) independent of (f) ($r_{tg \cdot f}$)	Degrees of freedom	Significance (Snedecor, 1946, p. 351)
Small cod maximum rations	0.8686	13	Significant at 1% level	0.7744	13	Significant at 1% level	–0.5607	13	Non-significant at 5% level
Large cod maximum rations	0.6822	13	Significant at 5% level	0.7831	13	Significant at 1% level	–0.5349	13	Non-significant at 5% level
Small cod intermediate rations	0.8767	12	Significant at 1% level	0.8471	12	Significant at 1% level	–0.5996	12	Non-significant at 5% level
Large cod intermediate rations	0.3519	15	Non-significant at 5% level	0.5347	15	Non-significant at 5% level	0.1335	15	Non-significant at 5% level

relate food used for growth to growth of the cod, eliminating food used for maintenance. However, figures indicating how maintenance requirements vary with different water temperatures in each 3-week period were not available from this experimental work.

The relationship between water temperature and growth was examined for all tanks and is shown in Table XII. Calculations showed poor correlation. The experimental work indicated that any correlation found between temperature and growth would likely be an indirect one working through food consumption and its resultant effect on growth.

DISCUSSION

WATER TEMPERATURE

Water temperatures in the Gulf of St. Lawrence have been discussed by Lauzier (1957a, 1957b). He found that seasonal and year-to-year variations do not follow any regular pattern. Lauzier (unpublished data) has shown that temperature fluctuations in Gulf of St. Lawrence water deeper than 30 fathoms are of the order of 3°C from about −1°C to 2°C, while in shallower waters fluctuations are greater. Charts of these variations in the Gulf of St. Lawrence (Lauzier, 1957a, b) indicate that very precise information on cod distribution by depth and area would be necessary in order to correlate the 1949–1959 growth changes (Fig. 7 and 8) with water temperature fluctuations. This type of information is not available.

Data on year-to-year trends in surface temperature in the region were examined for the possibility that long-term trends in water temperature in the Gulf might account for changes in food consumption, and ultimately, growth. Figure 20 shows a series of these temperatures for the months of May to August in the years 1937 to 1959 (Lauzier and Marcotte, unpublished data). Trend lines have been drawn in by these authors for the Grand River (Unit Area 4Tn, Fig. 1) and Entry Island (Unit Area 4Tf, Fig. 1) data. Here, although there is

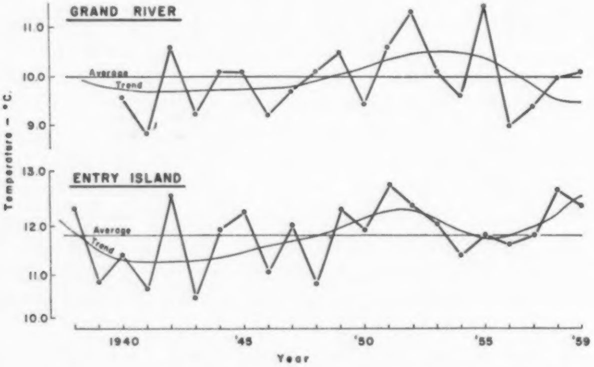

FIG. 20. Average surface-water temperature in the Gulf of St. Lawrence (May–Aug.). Curved lines show the trend (from Lauzier and Marcotte, unpublished data).

a tendency toward an upswing in the early 1950's at both places, the magnitude of the change (about 1°C) is not of the order that produced major changes in food consumption and resultant growth in our experimental work. Much greater fluctuations in water temperature would be required to produce growth variations of the magnitude shown in Fig. 7 and 8.

POPULATION DENSITY

Landings and catch per unit effort of cod for the years 1947 to 1959 from the Gulf of St. Lawrence population are relevant to cod population density in the area. Figure 21 (from work by Dr L. M. Dickie (ICNAF, 1961)) presents the pertinent facts.

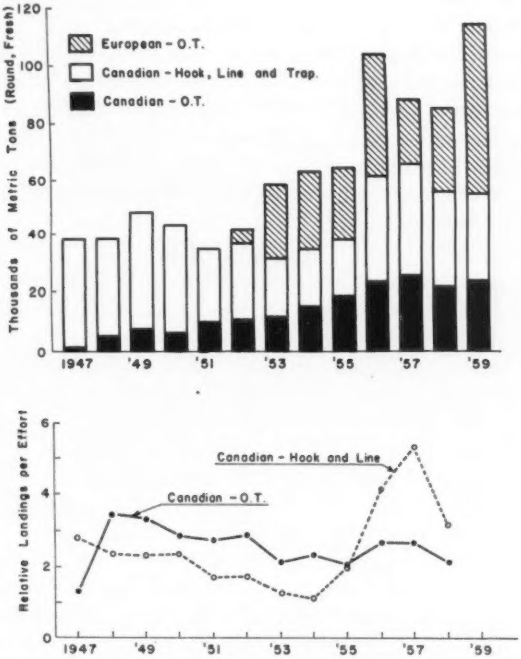

FIG. 21. Statistics of the fishery on the southern Gulf of St. Lawrence cod population. Data are from ICNAF (1961).

The upper graph in Fig. 21 shows landings by various fishing units from the cod population for the years 1947 to 1959. Total yearly landings in round fresh weight have almost tripled during the period, rising from about 39,000 metric tons in 1947 to over 115,000 metric tons in 1959. The breakdown by groups of fishing vessels indicates that much of the increase was due to rapidly mounting landings of the European trawling fleet from Spain, Portugal and France.

Their landings increased from nil in 1951 to 59,000 metric tons in 1959. Landings of the Canadian otter trawlers also increased greatly during the period from about 1000 metric tons in 1947 to 24,000 metric tons in 1959. This increase was almost all due to the great expansion of the small dragger fleets of northern New Brunswick and the Gaspé coast of Quebec. Canadian landings from hook-and-line and trap fisheries have fluctuated in a different manner, being relatively high in the 1947 to 1950 period, dropping from 1951 to 1955 and increasing again from 1956 to 1959.

The bottom graph in Fig. 21 shows relative density of the commercial-sized cod population based on statistics of the catch per unit effort of draggers and longliners engaged in the summer fishery in the southwestern Gulf of St. Lawrence. This fishery takes the bulk of the Canadian landings. Both otter-trawl and hook-and-line data show a steady drop in catch per unit effort of commercial sizes of cod from 1948 to 1953. The low is reached in 1955 for otter trawl and in 1954 for hook and line. At these points the indices for both gears rise, the hook-and-line index for 1957 being about five times that for 1954. Taking these indices as a measure of density of the cod population, and referring again to Fig. 7 and 15, it will be noted that 1954 and 1955 were years of relatively good growth and low density. In contrast, 1956 and 1957 data showed less growth and greater density.

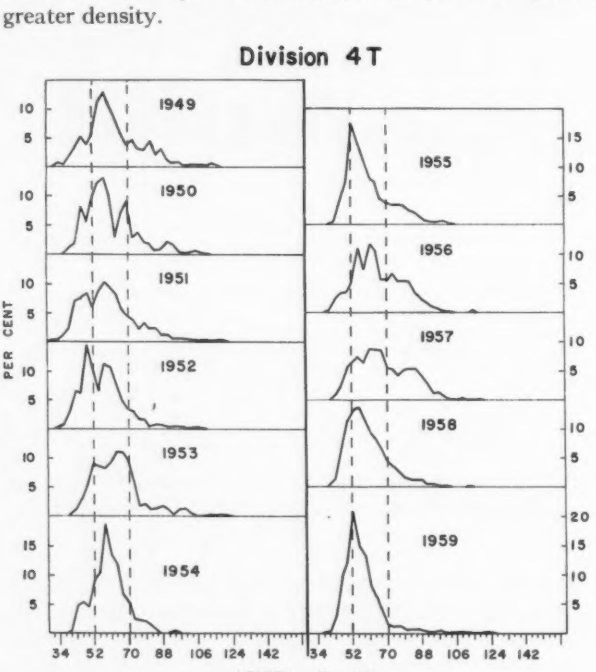

FIG. 22. Average length distributions of Division 4T cod for the years 1949–1959.

Data on length composition of the catch for the years 1949–1959 are plotted in Fig. 22 in order to relate average sizes of cod landed to density changes. Two lines based on average fish-plant cull were added to the figure, dividing off groups of small, medium and large size fish. Change over the years is most evident in the group of large (over 70 cm) cod. In years 1953 and 1954 when growth was increasing (Fig. 7) and density was low (Fig. 21) there were relatively few large cod available. On the other hand, in the years 1956 and 1957 the results of the good growth years are shown in an increasing availability of large fish. At this time the change in the proportion of these large-size cod in the area plus the increase in general density of the cod population would have further affected average growth because of changing competition for food.

AVAILABILITY OF FOOD

Powles (unpublished data) says of Gulf of St. Lawrence cod: "Small cod of 11 to 30 cm in length selected a diet of pelagic crustaceans, namely mysids, euphausids and amphipods. With increase in size cod adopted a more varied diet, in which fish and benthic invertebrates became increasingly important. At lengths over 70 cm, pelagic and bottom forms were taken in approximately equal volumes. Herring was the most important fish in the diet of large cod over 50 cm in length." Thus it was evident that one of the chief components of the diet of the cod of sizes shown in Fig. 7 was likely to be herring.

During the period of growth change described in previous sections, the herring population in the Gulf of St. Lawrence underwent an epizootic of a systemic fungus disease caused by the organism *Ichthyosporidium hoferi*. The course of the disease through the population is described by Sindermann (1958). Figure 23 is taken from his paper and shows locations of observed or reported mortalities of herring in the Gulf of St. Lawrence during the years 1954, 1955 and 1956. The effect of the disease on the Gulf herring population is most aptly described by Sindermann: ". . . dead fish (herring) were observed floating in shoals at the surface, were washed up on the shores of the Gulf, and were taken in the nets of otter trawlers . . . " and "Abnormal, lethargic, moribund herring could be seen inshore, particularly near the breakwaters, throughout the period. Deaths occurred sporadically in such inshore aggregations, the dying fish sinking to the bottom to become part of an extensive litter of herring carcasses". In his paper Sindermann estimated that at least one quarter of the mature herring in the Gulf were affected in 1955, and that some Gulf areas in 1954 had at least as severe mortalities. Observations for 1953 were not given, but for 1956 and 1957 the incidence was much lower.

With a large part of the herring population in this condition, the predator-prey relationship between cod and herring was altered markedly. Herring became much more easily available as food for cod, both on the bottom as dead fish and pelagically as slow-moving, moribund individuals. Casual observations by two Fisheries Research Board of Canada biologists, Dr F. D. McCracken and Mr P. M. Powles, in the summers of 1954 and 1955, indicated that cod stomachs were unusually full of herring.

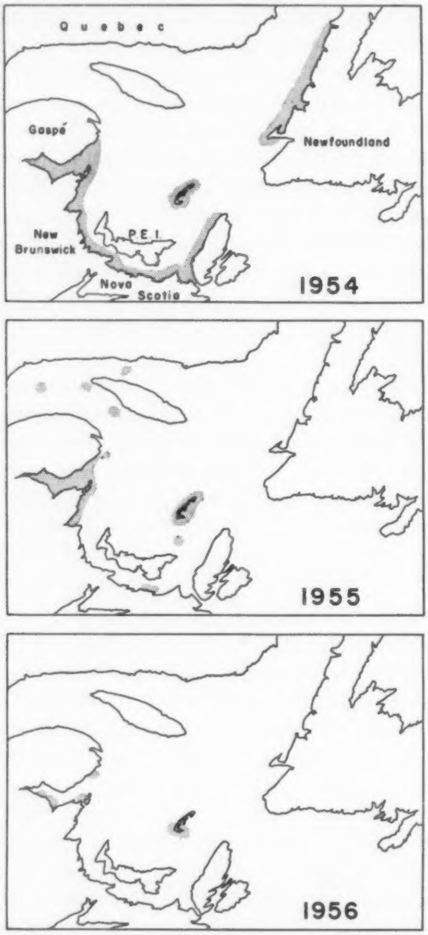

FIG. 23. Locations of observed or reported mortalities of sea herring in the Gulf of St. Lawrence. From Sindermann (1958). Localities of observations are shown by dots.

By use of data from Corbeil and Brunel (unpublished data) for 1951–1954, from Powles (1960) for 1955–1956, and from Jean (unpublished data) for 1957–1958, Fig. 24 was constructed to give an estimate of incidence of herring in cod stomachs over the years of growth change. The figure shows that percentage incidence of herring in each sampling year increases with the size of the fish as was noted by Powles (unpublished data). The peak of incidence of herring in cod stomachs for both the 51- to 70-cm fish and the group over 70 cm occurred in 1954. A general rise to this peak may be seen in the years 1951 to 1953, and a more rapid

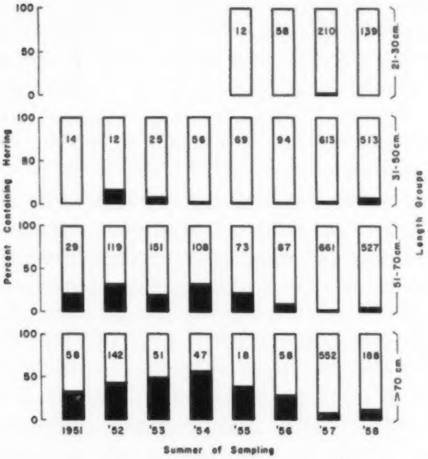

FIG. 24. Percentage of feeding cod from the southern Gulf of St. Lawrence whose stomachs contained herring. Exact length groupings for 1958 data are 20–29, 30–49, 50–69 and > 69 cm. Number of stomachs per sample are shown. Shaded areas indicate stomachs containing herring. Data are from Corbeil and Brunel (unpublished data) for 1951–54, Powles (1960) for 1955–56, and Jean (unpublished data) for 1957–58.

drop in the years 1955 and 1956 to a low in 1957 and 1958. Figure 7 shows that these were the sizes of fish with which the growth change was associated, and the rise in size-at-age certainly occurred during these years when herring were most easily available as food. Figure 15 gives the years 1954 and 1955 as those when growth was furthest above average, and these years are coincident with those in which Sindermann (1958) observed peaks in herring mortalities.

The drop in cod size-at-age in the years 1957–1959 (Fig. 7) could also be associated with the drop in incidence of herring as cod food (Fig. 24) and the reduction of Gulf herring stocks indicated by Sindermann. Thus, for this particular change in growth of Gulf of St. Lawrence cod, there is ample evidence of a change in availability of food both from observations on the effect of widespread disease on the prey species itself and the incidence of the prey in the stomach contents of the predatory organism. The data indicate that food availability was a prime factor contributing to the growth change.

CONCLUSIONS

Analysis of field collections of cod data established that cod growth varies both between populations and within the same population.

Analysis of laboratory experiments showed that food consumption increased with increasing availability of food. With an unlimited food supply, maximum food consumption was positively correlated with water temperature over a wide range of water temperatures (at least 2–12°C). Growth of cod increased with increasing food consumption. Examination of temperature–growth correlations indicated that growth of cod in tanks was only indirectly related to temperature through food consumption.

An increase in growth of cod in the southern Gulf of St. Lawrence was coincident with increased availability of herring, an important food of cod, high percentages of cod stomachs containing herring and a relatively low density of fish-eating sizes of cod in the area. A subsequent reduction in cod growth was coincident with a subsidence in the herring disease which greatly diminished the availability of moribund individuals as food, a reduction of herring stocks due to the disease, and a rise in density of cod stocks, particularly the larger members. Available water temperature data showed no variation that would greatly influence food consumption and resultant growth. It is concluded that changes in abundance and availability of the herring as food, plus changes in availability of food as a result of density changes in the cod population, were the prime factors responsible for the growth change.

SUMMARY

1. Methods of estimation of growth of cod resident in the waters over the continental shelf on Canada's east coast were examined. The main method used in this investigation was that of sampling commercial otter-trawl landings of cod for age and length. In order to investigate the validity of growth information from this source, the otolith method of age determination was tested. The validity of cod age determinations was confirmed by establishing that the formation of one hyaline and one opaque zone in the cross-section of the otolith is a yearly occurrence, by showing the consistency of relative strength of year-classes in the ages as determined from year to year, and by demonstrating the yearly production of one annulus in the otoliths of fish in length groups defined by the Petersen method.

2. Differences in growth of cod in the area were demonstrated both for widely-separated populations such as those in ICNAF Divisions 1F and 4W during the same years and in a single population, such as that resident in ICNAF Division 4T, among years. The latter growth change was studied in detail. The change itself was indicated by age and length parameters taken from commercial sampling, which showed a rise in mean length of age-groups from a low in 1951 to a high in 1955–1957 and a return to lower levels in 1958 and 1959.

3. It was suggested that the change in size-at-age might have been due to varying availability of cod normally caught only by the longline fishery over rough bottom. This fishery had been shown to take fish of larger average size than the otter-trawl fishery, and it was suspected that longlines

might select faster-growing, more voracious fish than the otter trawls. A comparative fishing experiment showed that on the same fishing grounds longlines took longer cod than the otter trawl, but age determinations showed them to be older also. There were no consistent differences in average size-at-age of cod between the two catches.

4. Annual increments on otolith cross-sections were measured to see if their relative sizes corresponded with good and poor growth years found by sampling commercial landings. The data from the two methods agreed as to good, poor and average growth years, with the years 1954 and 1955 showing up as the best years for growth.

5. Laboratory experiments were devised to assess some of the factors that might have an important influence on cod growth rates. Cod were kept under controlled conditions at the St. Andrews Station for over a year. Groups of small and large fish (initially 33–40 cm and 40–46 cm) were fed at maximum, intermediate and maintenance rates, while the water temperature in the tanks was allowed to fluctuate with normal seasonal variations found in the sea water from the area. Results showed that amounts of frozen whole herring needed to maintain cod at a constant weight for a year under these conditions were 1375 g for initially large cod and 1176 g for initially small ones. Conversion factors for food above maintenance requirements used for growth to cod weight were constant at 2.2 for large and small maximum-fed fish and 2.4 and 2.1 for initially large and small fish, respectively, on intermediate rations.

6. Some of the captive cod were in spawning condition over the latter half of the experimental period. Individuals were in the ripe stage in various months from March to September, and the attainment of this stage did not appear to be correlated with any one environmental factor. Loss of weight due to expulsion of spawning products was not taken into account in calculating food conversion factors, both because the loss was difficult to estimate and because it would be a normal occurrence in the wild population.

7. Calculation of correlation coefficients showed that, when the food supply was unlimited, food consumption increased with increasing temperature. Also, growth increased with increasing food consumption. Temperature was not well correlated statistically with growth.

8. Data on water temperature, cod density and availability of food were investigated for the Division 4T cod environment during the years of growth change. It was concluded that changes in abundance and availability of herring as a food organism plus changes in availability of food to individuals as a result of density changes among the larger members of the cod population were the prime factors responsible for the growth change.

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Polyphosphate Treatment of Frozen Cod. I. Protein Extractability and Lipid Hydrolysis[1]

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ABSTRACT

Tripolyphosphate dipped and undipped control paired cod fillets stored at −12°C showed no differences occurring on storage in extractable protein, lipid hydrolysis, or thaw drip. A higher yield of frozen and of thawed fillets was obtained from the dipped samples due to water uptake.

No specific effect of tripolyphosphate on thaw drip was obtained at the ionic concentrations used, but it is postulated that such effects might occur in the presence of about 1% salt.

INTRODUCTION

Various dehydrated and polymerized phosphates such as hexametaphosphate, pyrophosphate, tripolyphosphate, and adenosine triphosphate (ATP) have been used for some time now to improve moisture retention in frozen cheese (Guttenberg and Firgan, 1937), sausage meat and various meat products (Ellerkamp and Hannerland, 1952; Sherman, 1961; Hellendoorn, 1962). The literature is reviewed by Hellendoorn (1962) who concludes in part that tripolyphosphate and pyrophosphate are the most effective compounds, but that these must be used in combination with salt at the proper ionic concentration and pH, otherwise sodium chloride alone may be equally effective.

Bendall (1954) found a specific effect of pyrophosphate and ATP at high ionic strength in increasing swelling of minced muscle and also moisture retention on cooking. This effect was confirmed by Hellendoorn (1962) for tripolyphosphate as well, a compound with a phosphate structure similar to ATP.

Thus, in minced tissue such as sausage meat, moisture retention can be obtained by addition of these polyphosphate compounds. Presumably "weep", the free liquid or drip which tends to exude from whole fish fillets or cuts of meat, should also be lessened by such treatment. The swelling tendency in minced halibut muscle is maximum at about 0.5 to $1M$ NaCl (Tarr, 1942), about $0.5M$ for ground beef treated with NaCl (Wierbicki *et al.*, 1957), and at a somewhat higher concentration in larger pieces, 1 to $1.5M$ for whole herring (Reay, 1936).

However, immersion in water alone results in considerable uptake of solution which is then released rapidly during the first few hours of storage in the case of fillets (Castell, unpublished results), or yields cooking losses much greater than the undipped control (Wierbicki *et al.*, 1957).

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Presumably, the factors which influence weep and moisture retention in the cooked product also affect "thaw drip", i.e. the liquid sometimes released on thawing of frozen products. The mechanism is somewhat different since here the water released by melting of the ice crystals during thawing has to be reabsorbed by the protein. In ground beef, however, the addition of NaCl to an ionic concentration of 0.5 gave a similar maximum effect on both weep and thaw drip (Wierbicki *et al.*, 1957). Both Tarr (1942) and Holston and Pottinger (1955) find that at the pH of fish muscle, thaw drip is reduced to a minimum when the salt concentration in the muscle reaches about 1%. In relation to the use of phosphate compounds for modifying thaw drip, Heen (1953) mentions their use on fish, and a U.S. patent for "inhibiting the loss of moisture . . . of frozen fish flesh on thawing and cooking" has recently been granted (Mahon, 1962). Data are given for solution uptake and thaw drip in haddock treated with various phosphate salts, and some reduction in thaw drip was found over that of control samples similarly dipped in water.

Information was lacking, however, on the effect of such phosphate treatments, now increasingly used in the fishing industry, on the storage life of frozen fish and some experiments were undertaken to assess some aspects of this question, particularly the effect on protein extractability and lipid hydrolysis. Another report deals with its application in refrozen samples.

EXPERIMENTAL

Paired fillets of six market-size (3–5 lb), trawler-caught offshore cod (October 1962), iced on board 3–4 days, were used; one fillet served as a control while the other was treated. The fillets were given a 2-min dip in a 9% solution of sodium tripolyphosphate, the amount of dip solution used being about 7% of the weight of the fish to be dipped (Mahon, 1962). The fillets were individually weighed prior to and following treatment, packaged in 1-lb waxed paper cartons, and frozen in a horizontal plate freezer (Dole Freze-Cel) at a refrigerant temperature of approximately −37°C (−35°F.). After 1.5 hr they were removed from the freezer, sealed in polyethylene bags and stored at −12°C.

The frozen samples were analyzed in duplicate for extractable protein nitrogen (EPN) by the method of Dyer *et al.* (1950), using 22.5-g samples, unthawed, extracting 2.5 min with $0.6M$ NaCl ($0.003M$ $NaHCO_3$) in a Waring blendor (500-ml jar) fitted with a baffle plate, at a speed of 7000 rpm. The extracts were centrifuged for 30 min at 2000 rpm (820 × *g*).

Total lipid was extracted by the chloroform–methanol phase separation procedure (Bligh and Dyer, 1959). Before titration of the free fatty acids (FFA) the phospholipids were removed by passing the chloroform extract through a column of silicic acid. Results are calculated for an average fatty acid molecular weight of 300.

Thaw drip was determined by a procedure similar to that of Hartshorne and Puncochar (1951). A piece of frozen block, approximately 100 g, was placed on an inclined corrugated tray emptying into a funnel over a graduated cylinder, the whole assembly being enclosed in a tight-fitting polyethylene bag to prevent evaporation, and left to thaw in a cold room at 2–3°C (35–37°F) for 48 hr (Dyer *et al.*, 1962).

RESULTS

The fillets behaved differently in uptake of polyphosphate solution (Table I), three fillets absorbing 2.2–3.7% solution, the other three absorbing 6.5–9.0% (fillet No. 4—9%). This difference was also reflected in protein and lipid changes. The fillets which absorbed less solution showed a very rapid drop in protein extractable in salt solution, but it was less rapid in the other fillets, and FFA formation was also slower. In both groups, however, there was no difference between control and treated samples in either protein extractability or lipid hydrolysis. When FFA values for the treated samples are adjusted for the uptake of dip solution, the average difference from the control is 7% (+8% in fillet 2 and −15% in fillet 6), a difference about equal to the variation in the two samples from each fillet.

TABLE I. Extractable protein, lipid hydrolysis and thaw drip in sodium tripolyphosphate-dipped frozen cod fillets stored at −12°C.

Storage time at −12°C	0		3 weeks		11 weeks		17 weeks			
	C*	T	C	T	C	T	C	T	C	T
Fish no.	1		2		6		3		5	
Fillet weight, g		325		246		285		245		230
Dip uptake, %	–	2.2	–	2.8	–	6.7	–	3.7	–	6.5
EPN, %	2.14	2.09	0.70	0.71	1.33	1.31	0.94	0.90	1.01	1.27
	2.19	2.09	0.62	0.68	1.32	1.35	1.02	0.84	1.11	1.08
FFA, mg/100g	2.7	2.1	129	143	224	177	349	335	304	262
	1.9	2.2	147	148	220	177	350	321	291	271
Thaw drip, %					13	12	4.3	2.7	5.0	7.3

*C — control fillet.
T — treated fillet from the same fish.

The rate of lipid hydrolysis is in agreement with that obtained by Bligh and Scott (unpublished results), and also with that reported by Olley *et al.* (1962) for cod stored at −14°C, when allowance is made for the slight temperature difference.

Similarly, the decrease in EPN is about equal to that reported by Dyer and Fraser (1959) for cod stored at −12°C, the level having reached about 40% of the total protein after 17 weeks' storage. The rate quoted by Olley *et al.* (1962) is slightly more rapid, about 30–35% remaining after 17 weeks at a slightly lower temperature (−14°C). However, the value obtained in both the control and treated fillet of fish No. 2 is much lower than that usually obtained.

There seems to be no consistent effect on thaw drip attributable to polyphosphate treatment. Drip appears to be much less in both control and treated fillet No. 3 (low uptake of dip) than in Nos. 5 and 6 (high uptake). The magnitude of thaw drip, of course, is very dependent on the procedure used, but there is no indication of differences in the present results. It will be evident at once that the yield of both the frozen and the thawed product will be some 2–9% higher in the polyphosphate treated samples. However, the final yield of *thawed* treated product is about equal to or less than the weight of fish used, since the loss by thaw drip is greater or about equal to the liquid absorbed during treatment. The untreated product suffers a loss of thawed weight, in one case 13%. On balance, then, the benefit of the polyphosphate treatment lies in a yield of thawed product greater than the untreated product by about the amount of the thaw drip. The same effect can also be obtained by brining, as is often done in commercial practice. However, if the yield of the frozen product is considered, then the frozen weight of the treated samples is greater by some 2–9% than untreated fish.

Treatment of cod fillets for freezing thus increases the yield of the frozen and thawed product, with no detectable deleterious effect on protein "denaturation" or lipid hydrolysis.

DISCUSSION

The specific effect of pyrophosphate, ATP and tripolyphosphate on swelling and moisture retention in muscle tissues observed by Bendall (1954) and Hellendoorn (1962) is attributed to splitting of the fibrillar protein actomyosin into its components actin and myosin. Increasing the ionic concentration to about 1–1.5 with NaCl or other salts also dissociates the actomyosin (Ellis and Winchester, 1959) and promotes swelling, etc. The extra effect of the above polyphosphate compounds over that observed at equivalent ionic strength due to other salts must be due to some special effect on the dissociated products myosin and actin, which increases their moisture-holding capacity. The presence of sufficient additional salt is required to raise the ionic concentration to about 0.35 or above for pyrophosphate and to above 0.4 (preferably about 0.6) with tripolyphosphate to obtain the specific phosphate effect. This would have the advantage of providing conditions conducive to actomyosin dissociation but without the necessity of raising the salt concentration to unpalatable levels. Although these results have been obtained by measuring swelling and moisture retention on heating of minced or ground meat in the presence of excess liquid, it is likely that the conclusions will be applicable to thaw drip of whole pieces of muscle such as fish fillets.

Some comparative figures are available for haddock fillets, brined and also treated with sodium tripolyphosphate. Holston and Pottinger (1955) report an uptake of about 0.8 to 1% NaCl with a 20-sec dip in half saturated brine, and about 0.4% at 20% saturation. Taking a value of 0.24 (=1.4% NaCl (Dyer *et al.*, 1957)) as the ionic concentration of cod or haddock muscle, then the ionic concentration will be about 0.4 and 0.33 for the 50% and 20% dips, respectively.

On the other hand, Mahon (1962) using 7.5–12.5% dips of sodium tripolyphosphate obtained an uptake of about 0.2–0.4% in the presence or absence of 4% salt in the dip. Using Hellendoorn's figures for tripolyphosphate, the ionic concentration in the muscle will be about 0.24 +0.06 = 0.30 for 0.2% uptake and 0.36 for 0.4% uptake. The 4% salt dip would add about 0.4% NaCl, bringing the ionic concentrations to approximately 0.36 and 0.42. These levels are well below those found effective by Hellendoorn (1962) and Bendall (1954).

The results obtained by these authors on thaw drip, uptake of dip solution, and of resulting yield are compared in Fig. 1. It is seen that a 20-sec dip in half saturated NaCl gives about the same reduction in thaw drip as a 2-min dip in 7.5–12.5% sodium tripolyphosphate. It is readily apparent that the latter treatment was not more effective than salt alone because of the low total ionic concentration in the phosphate treated muscle.

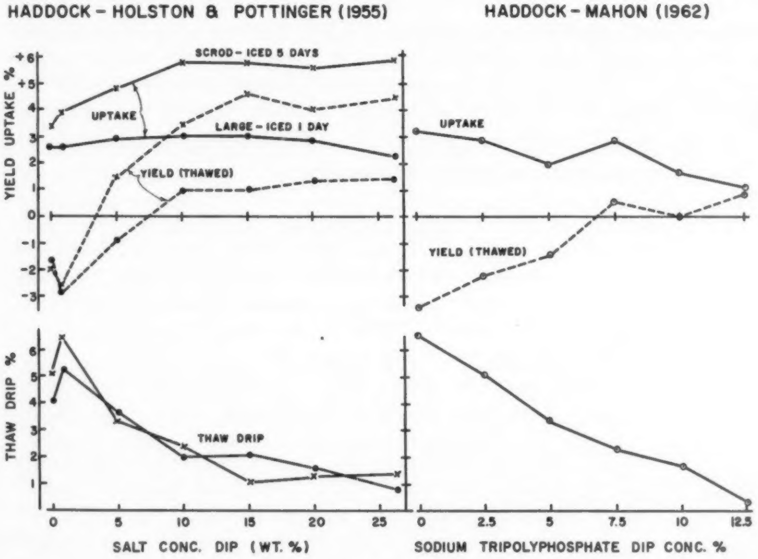

FIG. 1. Solution uptake, thaw drip and yield of thawed product in haddock fillets. Data from Holston and Pottinger (1955): Dipped 20 sec in NaCl brine, frozen and stored 3 weeks at −18°C. Data from Mahon (1962): Dipped 2 min in sodium tripolyphosphate, frozen and stored 34 days at −18°C. Yield is calculated from the difference between solution uptake and thaw drip.

Similarly, in the cod used in the present study, the ionic concentration would be too low to expect any special effect of the tripolyphosphate. The added sodium tripolyphosphate is about 0.2–0.5%, giving an ionic concentration of 0.3–0.4 in the muscle, and as the experimental results show, no effect on protein extractability or drip would be expected under these conditions.

It is concluded, therefore, that the optimum effectiveness of added tripolyphosphate in controlling thaw drip would be obtained if about 1% NaCl is also added to the fillets by a suitable brine dip. The interesting question of how such a treatment would affect the frozen storage life of the product and accompanying changes in proteins and lipids is still unanswered.

It will be noted also in Fig. 1 that the so-called control fillets dipped in water absorbed rather large amounts of fluid, much of which was later lost as thaw drip. In actual production lines, proper draining of the fillets between washings or conveying in "flumes" should eliminate at least a major part of this extra fluid (Dyer and Fraser, 1961).

The apparent effectiveness of brine or phosphate dips in reducing thaw drip is much lessened if comparison is made with undipped controls rather than with water-dipped controls. This effect explains the similarity of thaw drip values in untreated and treated samples in the present results with cod fillets (controls not dipped in water). In commercial practice, results may vary between these limits, depending on the degree of draining of the fillets and the time elapsing between washing, packing and freezing. While water-soluble protein, vitamins and minerals may be lost in thaw drip, this is not a problem when frozen fish are cooked without prior thawing, now a commonly recommended practice. During preparation of fish sticks from frozen blocks of fillets, little or no thawing of the fish portion occurs during the frying of the breaded stick; thus here also losses by thaw drip are becoming much less important.

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Neurosine, a New Oligopeptide Isolated from the Brain of Fish and Other Cold-blooded Vertebrates.
I. Identification and Partial Characterization[1,2]

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ABSTRACT

Analysis of fish brain for the dipeptide homocarnosine has led to the discovery of a new oligopeptide which is named neurosine. This substance contains γ-aminobutyric acid (GABA) and histidine residues. Most of the histidine present in a water soluble fraction from fish brain appears to be in the form of a tetrapeptide consisting of glycine, glutamic acid, GABA and histidine. Neurosine is ninhydrin negative, heat stable, and has a high R_f value when chromatographed in a butanol–acetic acid–water solvent system. The evidence at present suggests that this compound may be a cyclopeptide or contains a pyrrolidone or diketopiperazine ring structure.

Neurosine is present in high concentration in the brain of the killifish, *Fundulus heteroclitus*, approximately 0.1–1.0 μg of peptide per milligram of tissue. It also has been found in other bony fish, amphibians and reptiles but is absent in cyclostomes, elasmobranchs, birds and mammals. Neurosine is not present in any tissue but brain.

INTRODUCTION

The isolation of γ-amino butyrylhistidine (homocarnosine) from beef brain by Pisano *et al.* (1961) and the demonstration of its unique distribution in the brains of mammals by Abraham *et al.* (1962) suggested a survey of the brains of poikilothermic animals for this substance. During this investigation, however, it was found that almost all of the histidine residues present in an aqueous extract of poikilotherm brain tissue did not correspond to histidine or to the dipeptides homocarnosine, carnosine, or anserine (Hosein and Smart, 1960). Almost all of the imidazole residues in fish brain extract were present in a single chemical moiety which had a high R_f value when chromatographed in a butanol–acetic acid–water system and which was ninhydrin negative. The isolation, characterization and distribution of the unknown substance in various vertebrates are reported.

MATERIALS AND METHODS

The following animals were used for extraction and identification of brain imidazoles: mouse, *Mus musculus*; chick, *Gallus* sp.: turtle, *Pseudemys floridana*; chameleon, *Anolis carolinensis*; grass frog, *Rana pipiens*; bullfrog tadpole, *Rana*

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catesbeiana; salamander, *Triturus viridescens*; common killifish, *Fundulus heteroclitus*; striped killifish, *Fundulus majalis*; goldfish, *Carassius auratus*; flounder, *Pseudopleuronectes americanus*; mackerel, *Scomber scombrus*; formosa molly, *Mollienisia formosa*; triple tail, *Lobotes surinamensis*; yellowtail snapper, *Ocyurus chrysurus*; sea lamprey, *Petromyzon marinus*; dogfish, *Squalus acanthias*.

Extraction Procedure

Brains were obtained by immediate dissection after snipping the spinal cord of the experimental animal. After weighing, the brain tissue was quickly homogenized in a ground glass homogenizer in a solution of 95% ethanol and $0.1N$ HCl (1:1) to stop enzymatic activity. About 50 mg of tissue was homogenized in 1 ml of solution and extracted for 2 hr at 6°C. The homogenate was then centrifuged and the clear supernatant used for further analysis. Approximately 5–20 μl of this solution is needed to give a positive imidazole test after chromatographic separation. The new oligopeptide is stable for 1–2 months in this solution when stored at 6°C.

Chromatography

Solvent systems: 1-butanol, acetone, water (10:10:5) (Hardy *et al.*, 1955). Top layer, 1-butanol, acetic acid, water (4:1:5) and 1-butanol saturated with $1N$ HCl (Erspamer, 1955).

Developing reagents: (Block *et al.*, 1958).

Ninhydrin reagent for amino acids and peptides: 0.1% in acetone or 1-butanol. Heat to 100°C for 1–2 min.

Ehrlichs reagent for indoles: 2.0% p-dimethylamino-benzaldehyde in 95% ethanol. After spraying, place in HCl vapor for 5–30 min.

o-phthalaldehyde reagent for glycine and histidine: 0.2% o-phthalaldehyde in acetone. After spraying, heat to 50°C for 10 min (Curzon and Giltrow, 1954).

Pauly test for imidazoles: solution (A), 25 ml of 5% $NaNO_2$ at 0°C added to 5 ml of sulfanilic acid solution (0.9 g of sulfanilic acid + 9 ml of concentrated HCl diluted to 100 ml with water). Solution (B), 5% aqueous Na_2CO_3. Spray with solution (A) and while still damp with solution (B) (Ames and Mitchell, 1952).

Paper: Whatman #1.

Hydrolysis

After separation by chromatography and elution of the unknown imidazole compound, an aqueous solution containing approximately 1 μg per 5 μl was incubated at 100°C with an equal volume of concentrated HCl for 6–8 hr. Incubation was carried out in sealed glass tubes or ground glass stoppered test tubes. From 10 to 40 μl of the hydrolysis mixture and ethanol–HCl washings were chromatographed for amino acid analysis against known standards given similar treatment. Enzymatic degradation of the unknown was accomplished by pre-incubating a 50-mg brain sample homogenized in 0.2 ml of water for 1 hr at 26°C prior to extraction with ethanol–HCl solution.

RESULTS AND DISCUSSION

ISOLATION AND DISTRIBUTION

The unknown imidazole compound was first isolated from an ethanolic extract of the brain of the killifish, *Fundulus heteroclitus* and identified by chromatographic analysis in 1-butanol saturated with 1N HCl and 1-butanol–acetic acid–water solvent systems. Attempts to isolate this material from muscle, heart and liver tissue of this animal were unsuccessful. In Table I, some of the properties of this unknown are compared to several standard imidazole compounds.

TABLE I. Chromatographic and chemical properties of several imidazole compounds.

Compound	R_f in solvent system[a] 1	2	3	Ninhydrin color	Pauly test
Unknown imidazole	0.103	0.299	0.206	none	+
Homocarnosine[b]		0.092	0.040	purple-violet	+
Carnosine		0.061	0.056	blue	+
Norcarnosine		0.050	0.042	yellow	+
Histidine		0.081	0.104	grey-blue	+

[a] Solvent systems: 1 (1-butanol saturated with 1N HCl)
2 (1-butanol–acetic acid–water, 4:1:5)
3 (1-butanol–acetone–water, 10:10:5).

[b] Isolated from mouse brain.

TABLE II. Distribution of the new brain imidazole compound among various animal species.

Superclass	Class	Species	New brain imidazole[a]	New brain imidazole as approximate % of extractable brain imidazole compounds[b]
Tetrapoda	Mammalia	mouse	–	0
	Aves	chick	–	0
	Reptilia	turtle	+	30
		chameleon	+	30
	Amphibia	grass frog	+	60
		bullfrog (tadpole)	+	95
		salamander	+	95
Pisces	Osteichthyes	common killifish	+	95
		striped killifish	+	95
		goldfish	+	95
		formosa molly	+	95
		flounder	+	95
		mackerel	+	95
		triple tail	+	95
		yellowtail snapper	+	95
	Chondrichthyes	dogfish	–	0
	Agnatha	sea lamprey	–	0

[a] Identified chromatographically in a 1-butanol–acetic acid–water solvent system (4:1:5) against a killifish, *Fundulus heteroclitus*, brain imidazole standard.

[b] Based on a neurosine standard developed by Pauly's reagent.

Since the unknown imidazole compound represented almost all of the imidazole residues in the extract of killifish brain, and since this substance was not homocarnosine, a survey of the brains of animals belonging to fish, amphibian, reptile, bird and mammalian species was undertaken. In Table II the results of this investigation and the distribution of the new brain imidazole compound are reported.

HYDROLYSIS OF UNKNOWN IMIDAZOLE

When a freshly dissected brain of the killifish is homogenized in 0.2 ml of distilled water and incubated at 26°C for 1 hr prior to extraction with ethanol–HCl solution it is found that all of the fast moving imidazole unknown is converted to a slow moving substance which is ninhydrin positive and with an R_f value near that of histidine. In addition, there is an increase in the amount of glycine and γ-aminobutyric acid (GABA) present in the crude extract.

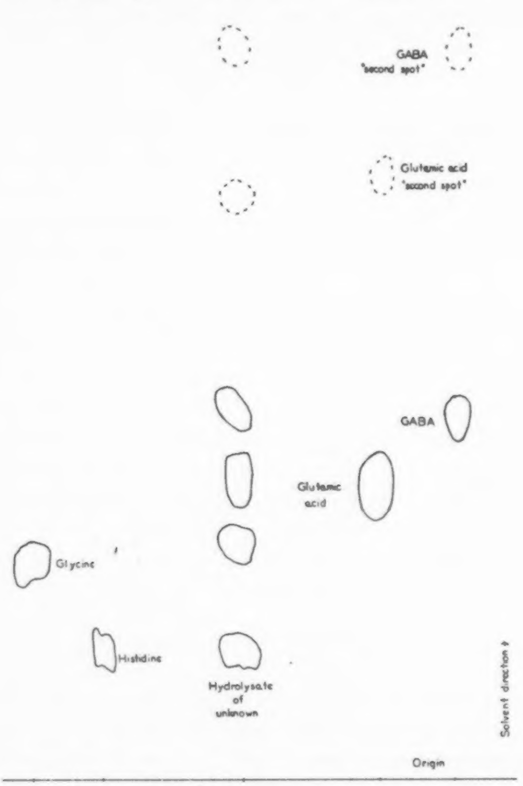
GABA
"second spot"
Glutamic acid
"second spot"
GABA
Glutamic
acid
Glycine
Histidine
Hydrolysate
of
unknown
Solvent direction
Origin

FIG. 1. Chromatogram (tracing made from original) of amino acid residues (developed with ninhydrin reagent) after hydrolysis of unknown imidazole compound compared to standard amino acids. Solvent: 1-butanol–acetic acid–water (4:1:5).

Hydrolysis of a chromatographically purified, eluted ninhydrin negative sample of the brain imidazole in 6*N* HCl at 100°C for less than ½ hr results in the complete conversion of the unknown to a single ninhydrin positive substance with an R_f in 1-butanol–acetic acid–water (4:1:5) near that of histidine. Further hydrolysis for 6 to 8 hr under these conditions produces residues of histidine, glycine, glutamic acid and GABA at estimated microgram quantities in the ratio of 1.0:0.2:0.8:1.0, respectively (Fig. 1). Indole residues which would be destroyed during hydrolysis have been shown to be absent by a negative Ehrlichs test for the unknown imidazole. These results indicate that the unknown is, therefore, an oligopeptide. In Table III the properties of the amino acid residues formed on hydrolysis of the unknown brain imidazole are compared to those of standard amino acids.

TABLE III. Comparison of amino acid residues in the hydrolysis mixture to amino acid standards.

	R_f in solvent(1)[a]	"Second spot" formation[b]	R_f in solvent(2)[c]	Ninhydrin color[d]	Pauly test	o-phthalaldehyde test
Hydrolysis residue #1	0.085		0.081	G B	+	+
Histidine standard	0.081		0.079	G B	+	+
Hydrolysis residue #2	0.192		0.157	P V	–	+
Glycine standard	0.191		0.151	P V	–	+
Hydrolysis residue #3	0.280	0.535		V	–	–
Glutamic acid standard	0.275	0.561		V	–	–
Hydrolysis residue #4	0.334	0.681		P V	–	–
GABA standard	0.334	0.681		P V	–	–

[a] Solvent (1) 1-butanol–acetic acid–water (4:1:5).
[b] Amino acid esters easily formed in alcoholic-HCl solutions (Koch and Hanson, 1953).
[c] Solvent (2) 1-butanol–acetone–water (10:10:5).
[d] G (grey), B (blue), P (purple), V (violet).

POSSIBLE STRUCTURE OF THE UNKNOWN BRAIN OLIGOPEPTIDE (NEUROSINE)

The absence of a ninhydrin response and the high R_f value in an acid solvent system indicates that there are no free amino groups and suggests that neurosine may be a cyclopeptide or contain a ring structure. Of the several possible cyclopeptide structures, a few may contain homocarnosine residues. The presence of glutamic acid and glycine residues suggests two possible ring configurations that would satisfy the experimental results. In the case of a terminal α-glutamyl residue, it is possible to form a five-membered pyrrolidone ring which is ninhydrin negative (Wiltshire, 1953). Fastigiatin, a tripeptide of three glutamine residues isolated from a marine alga by Dekker *et al.* (1949) contains such a structure. However, the pyrrolidone ring tends to be formed under the conditions of extraction and acid hydrolysis (Ellfolk and Synge, 1955). This does not agree with the experimental evidence which indicates that neurosine is converted from a fast moving ninhydrin-negative substance to a slow moving, ninhydrin-positive material after only 30 min in 6*N* HCl at 100°.

A second possible configuration involves the formation of a six-membered diketopiperazine ring between glycine and glutamic acid. This type of ring is easily broken by acid hydrolysis to form, in the case of diketopiperazine itself, first the dipeptide glycylglycine and then two glycine residues. This second explanation is the more plausible based on the results of hydrolysis. The isolation of homocarnosine from cow brain (Pisano *et al.*, 1961) and pig, dog and cat and human brains (Abraham *et al.*, 1962) suggests that the homocarnosine residue may exist in the oligopeptide isolated from fish, amphibians and reptiles also.

While the function of the imidazole oligopeptide is at present unknown, the very high concentration of this substance found only in brain tissue suggests that it may play an important role in neural function. The concentration of neurosine in fish and amphibian brain tissue is from 10 to 100 mg per 100 g of tissue which is significantly higher than that of homocarnosine (0.7 mg/100 g of brain) found by Pisano *et al.* (1961) for beef brain.

The presence of neurosine in brain is important when it is considered that an enzymatic mechanism exists in brain tissue to cleave this compound into its four amino acid residues, three of which are potent or potential mediators of nerve activity. γ-aminobutyric acid is a direct product of hydrolysis, and the presence of glutamic acid decarboxylase and histidine decarboxylase in brain and other tissues can form additional GABA and histamine, respectively (Dixon and Webb, 1958).

The apparent phylogenetic distribution of neurosine and of homocarnosine in brain is striking. The point of separation between these peptides seems to occur during the evolutionary transition from a poikilothermic to a homiothermic condition. In the homiotherms, birds and mammals, the oligopeptide is absent. In the poikilotherms, fish and amphibians, it represents most of the extractable imidazole residues. The reptiles form a transition group in this respect in that they are capable of withstanding desiccation and thus can seek out hot-dry environments to elevate their ambient body temperature. In the reptiles, the oligopeptide represents only about 30% of the extractable brain imidazoles, the remainder of which is chromatographically indistinguishable from and may indeed be homocarnosine.

In addition, the distribution of neurosine may also be correlated with a transition from a fully aquatic to a completely terrestrial mode of life. In the aquatic forms, fish and aquatic amphibians such as the newt and the tadpole, neurosine is the primary brain imidazole compound. In the adult frog it represents only 60% of the soluble imidazoles, and in the more terrestrial reptiles only 30% of the brain imidazole residues. In the fully terrestrial forms, neurosine is absent.

CONCLUSIONS

The oligopeptide, neurosine, which has been isolated from the brains of poikilotherms, may contain a homocarnosine residue and may also be functionally related to homocarnosine present in homiotherm brain tissue. Its distribution suggests the possibility of biochemical evolution in vertebrates along phylogenetic lines, with reptiles representing an intermediate form.

Work is now in progress to confirm the chemical structure of neurosine, to survey other animal classes for its distribution in nerve tissue and to determine its pharmacological properties.

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Water-Thawing of Frozen Cod Blocks[1]

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ABSTRACT

Thawing time for frozen blocks of dressed cod (*Gadus morhua*) was investigated. Observations were made on the effect of size of individual fish, water recirculation rates, water temperature, block height, thickness and orientation, separation of individuals from the block at some intermediate time, and the use of sodium alginate to promote quicker thawing. Over the range investigated, the first two variables did not affect thawing rate; the remainder were of importance. Blocks 4 and 4½ inches (102 and 115 mm) thick, weighing 75–80 lb (34–37 kg), were separated and thawed in 60°F (15.5°C) water in about 3 and 4½ hr, respectively. These times were equal to or better than the times for freezing between plates under best local commercial conditions. In comparison, similar blocks can be thawed in about 1½ hr in a commercially available dielectric thawer. Thawing blocks of dressed fish in recirculated water thus merits consideration with other methods.

INTRODUCTION

In order to use frozen "rough"[2] fish for blocks or packages of finished fillets suitable for the North American market, the fish must be thawed, processed and refrozen at some stage following initial freezing. Since blocks of frozen rough cod (*Gadus morhua*) (Fig. 1) used in tests by Dyer *et al.* (1962) on the *storage quality* of the refrozen fish were to be thawed in water, it was decided to obtain reproducible, and if possible, reasonable thawing rates and to record features of the thawing process. This paper reports thawing data and times. In general, results indicate that recirculated tap water can be used to thaw blocks of cod sufficiently fast to merit its consideration with other methods of thawing these and similar fish.

Little detail has been published on thawing *blocks* of rough frozen cod in water, hence some of the theoretical and practical considerations which have bearing on the speed of thawing and on the relative speeds of thawing and freezing are treated in this work. Stiles (1922), noting that thawing is a relatively slower process than freezing, illustrated advantages of thawing in water over thawing in air. Young (1941, 1942) found that the rate of thawing had only a minor effect on the quality of halibut and salmon steaks in subsequent storage.

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[2] Term used here to identify gutted and headed or "dressed" cod. Similar fish, frozen in blocks, are sometimes referred to in the literature as "whole fish".

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Reay *et al.* (1950) also reported that variations in the rate of thawing found in actual practice made no material difference in the palatability or texture of the thawed product. They found that thawing in water was especially suitable for whole fish, and that 60°F (15.5°C) was the highest temperature to be recommended for any of the thawing media tried. Magnusson and Hartshorne (1952) reported on the water-thawing method used with brine-frozen *individual* whole cod and haddock used to provide raw material for finished refrozen fillets. They showed that the borderline between the frozen and thawed layers was between 30 and 32°F (−1.1 and 0°C), and that the time for thicker fish to thaw was disproportionately longer than that for thinner fish of the same weight (in agreement with Reay *et al.*, 1950), and that the rate of water circulation above 1 ft/min (5×10^{-3} m/sec) had only a minor effect.

With the development of vertical plate freezers for dressed fish (Yule and Eddie, 1953; Eddie, 1955; White Fish Authority, 1957), and the introduction of freezing ships, much more attention has been focused on thawing methods. Research findings on the dielectric-thawing method have been published by Jason and Sanders (1959, 1962a, 1962b). Their papers record some data on comparative air-thawing and water-thawing methods as well. Commercially available dielectric equipment effectively thaws both dressed and skins-on fillets of cod while maintaining the uniform temperatures desired in the product (MacCallum, 1963). In general, highest surface temperatures are about 40°F (4.4°C). Waterman (1963) has reviewed practical methods (air, water and dielectric) of thawing large blocks of frozen whole fish. Currently, air, water and electrical conduction methods are being investigated at the Torry Research Station (Reay, 1962; Sanders, 1963) while water, dielectric and microwave methods are being examined by scientists of the Fisheries Research Board of Canada and the U.S. Fish and Wildlife Service's Gloucester Laboratory. Industry in Great Britain, now making use of frozen whole eviscerated fish for filleting, has found the air-thawing method attractive from the standpoint of low costs, handling, and technological suitability. A few installations are now in use and others are planned.

EXPERIMENTAL

Apparatus and Equipment

The thawer used in these experiments (Fig. 4) consists of a water-tight rectangular plywood box finished in polyester resin and measuring about 26 × 59 × 23 inches (660 × 1500 × 584 mm) inside. It is equipped with a water-level control and means to recirculate tap water used for thawing. The temperature of the recirculated water can be controlled by means of a $\frac{1}{2}$°F ($\frac{1}{4}$°C) differential temperature controller which operates a solenoid valve, the latter being placed in a warm-water supply line. Initial and any necessary subsequent cooling of water is accomplished by introducing crushed ice or cold tap water into the tank. The capacity of the thawer is four 18 × 36 × 4$\frac{1}{2}$-inch blocks (457 × 914 × 115 mm) (Fig. 1). Blocks measuring 14 × 42 × 4 inches (356 × 1070 × 102 mm) or 22 × 42 × 4 inches (559 × 1070 × 102 mm) can be accommodated easily, as can portions of blocks of any of these sizes. Portions of full-

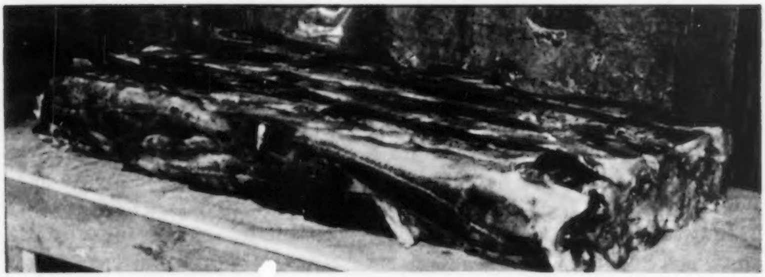

FIG. 1. An 18 × 36 × 4½-inch (457 × 914 × 115-mm) 90-lb (41-kg) block of dressed cod after removal from the vertical plate freezer.

sized fish blocks were placed on dummy wooden blocks to preserve constant flow conditions of the water in all cases. Guides were placed in the bottom of the tank or on top of the wooden blocks to separate the fish blocks which stand on their edges. The guides provide for a flow of water under the supporting edges of the fish blocks. The space between 4½-inch-thick blocks and between the blocks and sides of the tank is about 1½ inches (38 mm). Nozzles are placed to direct an even flow of water between, under, and over the blocks and between the blocks and the sides of the tank. Water in the tank is maintained at a level to cover the blocks. The recirculated water enters one end of the tank just below water level and leaves the tank near the bottom at the other end. The velocity can be varied by changing the pumping rate or the space within the thawer into which a given quantity of water is pumped per minute. Velocities of 4 and 8 ft/min (2×10^{-2} and 4×10^{-2} m/sec) across the surface of the fish blocks were provided using a centrifugal pump (2½-inch suction reduced to 1¼-inch) direct connected to a 2-hp, 3-phase, 220-volt, 1750-rpm, A.C. motor. The space normally available in the thawer was reduced to provide the higher velocity. A ½-hp, single-phase, 110-volt, 1725-rpm electric motor was used with a belt drive to operate a centrifugal pump (1½-inch suction) used to deliver water across the blocks at a velocity of 0.8 ft/min (4×10^{-3} m/sec).

Temperatures within the blocks in the thawer were recorded using copper-constantan thermocouples with a strip chart multi-point recorder. Connections between the thermocouple leads and instrument panel were made using copper-constantan plugs. Depending upon requirements, one or two sets of 12 thermocouples each were used in the thawing runs. The temperature of fish at time of separation from the blocks was recorded by thermocouples or was measured using a mercury-filled glass thermometer. Water temperature was both recorded and read from a thermometer. The temperature of each fish judged ready for removal from the water was read from a thermometer.

Two procedures were used for inserting thermocouple leads. First, the leads were placed in the fish at time of packing for freezing. These were used to develop both freezing and thawing curves. Other blocks, frozen without thermocouples, were drilled to the desired depth using a ⅛-inch or $\frac{3}{16}$-inch bit. One thermocouple lead was inserted in each hole and the hole was filled with fresh water which froze, holding the lead in position. Such blocks were returned to low temperature storage for equilibration of temperatures. No significant difference in rates of thawing was noted using the two techniques. The former ensured placement of the lead in the thickest section of a fish but did not contribute to more accurate positioning of the lead with respect to edges of the block than did the latter method.

PROCEDURES

Multiple thawing runs were conducted to obtain data on the various conditions which might affect thawing rates, i.e.: (a) fish size; (b) water recirculation

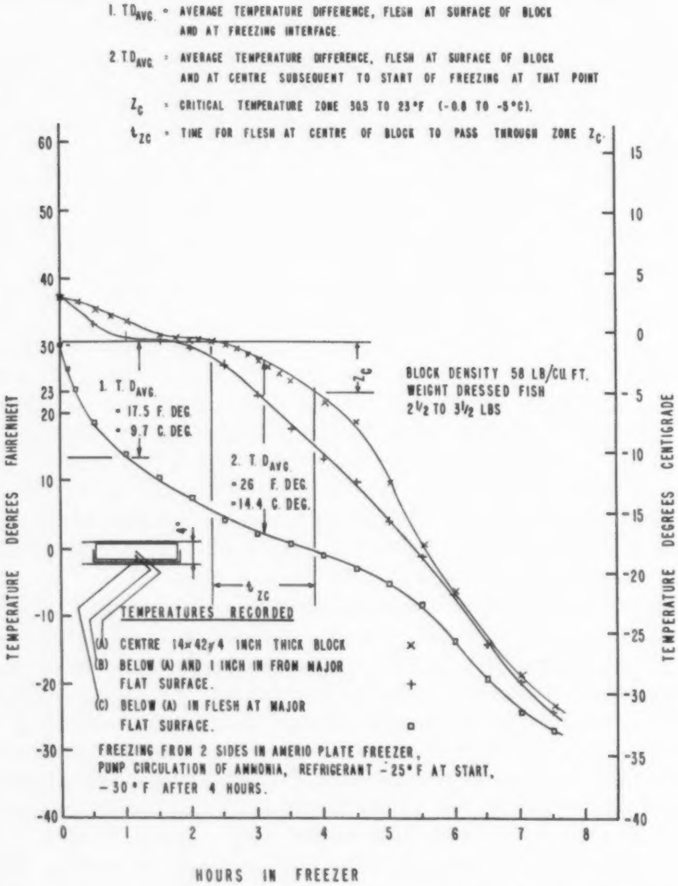

FIG. 2. Freezing curves for points at the centre, midway between the centre and the surface, and at the surface of 14 × 42 × 4-inch (356 × 1070 × 102-mm) blocks of dressed cod frozen in waxed paper packages and aluminum trays between horizontal freezer plates.

rate; (c) water temperature; (d) block height; (e) block thickness; (f) orientation of blocks in thawer; (g) separation of fish in blocks prior to complete thawing; (h) immersion of fish in a sodium alginate solution prior to freezing.

With one exception all data were obtained using blocks of headed, eviscerated pre- or post-rigor frozen cod having a density of about 53 lb/ft^3 (850 kg/m^3). The exception occurred with small inshore (trap) cod, frozen after storage for 24 hr in ice. These blocks had a high density of 58 lb/ft^3 (930 kg/m^3). Freezing and thawing data on these blocks are given in Fig. 2 and 3, respectively.

To provide samples for the tests, numerous blocks of 95–100 lb (43–45 kg) total weight were frozen in sections. This was accomplished by inserting aluminum alloy strips measuring about $35\frac{1}{2} \times 4 \times \frac{1}{4}$ inches (900 × 102 × 6.3 mm) at the required elevations in the stations of the vertical plate freezer while the fish were being packed. This involved little more than routine labour and for practical purposes no loss in freezer capacity. The blocks were separated easily at the aluminum strips to produce the various shallow-height blocks required for thawing experiments. Full-size blocks required for the tests were frozen without strips.

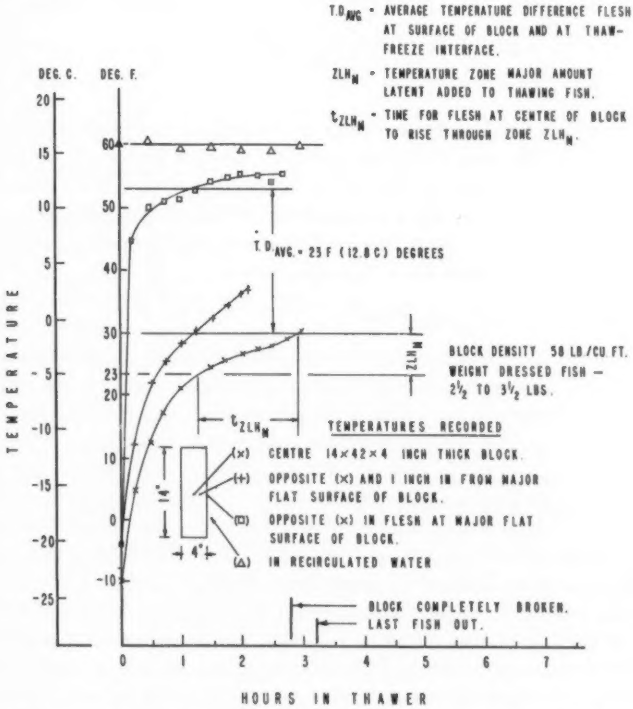

FIG. 3. Curves for thawing blocks of frozen cod (described in Fig. 2) in 60°F (15.5°C) tap water recirculated at a velocity of 4.0 ft/min (2×10^{-2} m/sec) in the thawer.

DEFINITIONS

"Removal temperature", as used in this paper, is close to 30°F (–1.1°C). At this temperature, cod are mostly but not entirely thawed. For purposes of this study, any fish reaching this temperature and not securely frozen to other fish was considered ready for transfer to the filleting line or to temporary iced storage. Fairly frequently it was found upon separation of a block that some fish were colder than others being monitored. Hence time–temperature relations often show monitored fish (Fig. 3, 6, 8) to be warmer than last-to-thaw individuals. Decision as to the time the last-to-thaw fish was ready for removal was a matter of the investigators' judgment (see Fig. 3, 6, 7 and Tables I–III).

The upper limit of temperature zone ZLH_M, in which the major amount of latent heat is added to the fish is assumed to be 30°F (–1.1°C) (Fig. 3, 6). The lower limit assumed is 23°F (–5°C).

Z_C in Fig. 2, a zone almost identical to ZLH_M in terms of temperature limits, but encompassing a wider latent heat range, has an assumed upper limit of 30.5°F (–0.8°C), a lower limit of 23°F (–5°C) and, conforming with popular usage, is called the critical temperature zone.

RESULTS

FISH SIZE

No significant difference was noted in the overall thawing times for separate blocks of equal weight and thickness and comprised of uniformly large as against uniformly small cod. This appeared to hold for fish of 2–6 lb gutted, headed weight. While the cod in blocks comprised of larger fish took longer to thaw once they were separated, these fish could be separated from one another sooner than could smaller individuals.

EFFECT OF RECIRCULATION RATES

Considerations of heat transfer showed that for water temperatures as warm as 60°F (15.5°C), conductance through the thawed muscle was the limiting factor, not heat transfer at the fish–water boundary. Thus it was demonstrated experimentally that low and moderately low water velocities of 0.8, 4 and 8 ft/min (4×10^{-3}, 2×10^{-2} and 4×10^{-2} m/sec) over the surface of a 4½-inch thick block did not produce appreciable difference in thawing rates (Table I). Individual fish in blocks weighing approximately 95–100 lb (43.2–45.4 kg) and exposed to water at 45°F (7.2°C) recirculated at the above velocities were ready for separation and for filleting at approximately equal times. Density of pack was about 53 lb/ft^3 (850 kg/m^3) and individual dressed fish weighed 3–4 lb (1.4–1.8 kg). A similar conclusion was reached for undisturbed blocks weighing 86–88 lb (39–40 kg) as indicated by measuring thermocouples and by the time required for last-to-thaw fish weighing 2.5–4 lb (1.2–1.8 kg) to reach removal temperature. Again, using 72- and 75-lb (33- and 34-kg) blocks, no saving in time to block breakup or reduction in thawing rates in the undisturbed block was evident when individual fish of 4–6 lb (1.8–2.7 kg) dressed weight were thawed.

TABLE I. Experimentally-determined times to warm last-to-thaw block-frozen dressed cod from 0°F (−18°C) to break-up state and to removal temperature in relation to velocity of recirculated thawing water at 45°F (7.2°C) (block size: 4½ inches thick by 36 inches long; block height proportional to weight).

			Actual time in hours			
			Individual fish separated at intermediate time		Block not separated during thawing	
Water velocity		Block weight	Separation at	All fish removed	As indicated by thermocouples	As shown by examination upon breaking block
ft/min	*m/sec*	*lb*				
8.0	4×10^{-2}	96	5.5	7.3	–	–
8.0	4×10^{-2}	88	–	–	8.0	9.5
4.0	2×10^{-2}	95	4.0	5.5	–	–
4.0	2×10^{-2}	75	4.0	5.2	–	–
4.0	2×10^{-2}	75	–	–	7.2	9.0
0.8	4×10^{-3}	75	4.3	6.7	–	–
0.8	4×10^{-3}	72	–	–	8.0	9.0
0.8	4×10^{-3}	99	5.0	7.2	–	–
0.8	4×10^{-3}	86	–	–	7.2	9.2

The conclusion that similar rates of thawing were achieved at the different water velocities is supported by the record of temperatures within and at the surface of the blocks. Temperatures recorded in the flesh at the surface, halfway between the surface and the centre, and at the centre of 72-, 86- and 88-lb (33-, 39- and 40-kg) blocks are shown in curves A, B and C of Fig. 5. It will be noted from the spread of points that differences could not be distinguished in the thawing rate resulting from water velocities of 0.8 and 8.0 ft/min.

With low-velocity water (0.8 ft/min) there was almost no frothing when blocks of trawler-caught cod were thawed. Similar samples thawed in water recirculated at 4 ft/min contributed to cloudiness in and scum on the water. At the lower velocity entrained material could settle to the bottom of the tank.

TEMPERATURE GRADIENTS DURING THAWING

The curves in Fig. 3 and 6 show changes in temperatures with time at the surface, midway between the surface and centre, and in the centre of cod blocks of two sizes and of two densities. The flesh at the surface of the block thawed almost immediately and the temperature rose rapidly to a high plateau. The flesh midway between the surface and centre of the block was thawed in about 1 hr. Thus flesh on the outside of an undisturbed block will be wholly or partly at an elevated temperature for a considerable period, making it advisable to maintain water temperatures as low as conducive to satisfactory thawing.

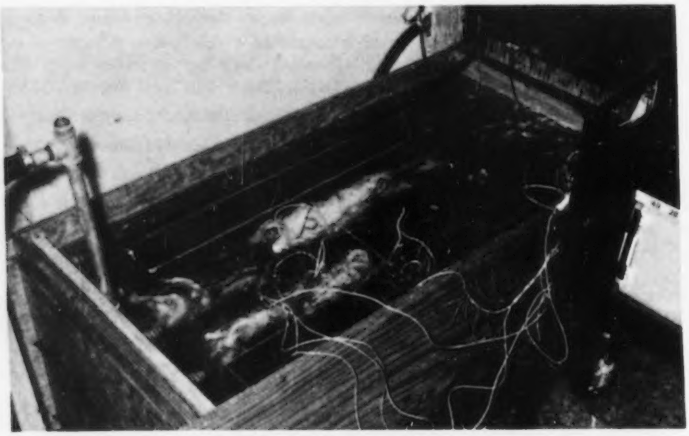

FIG. 4. Two 95-lb (43-kg) blocks of dressed cod are seen in the water thawer.

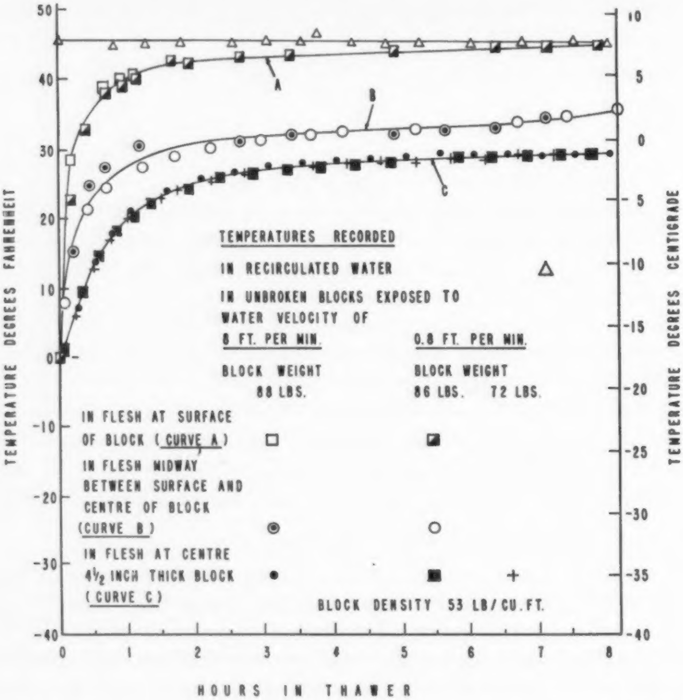

FIG. 5. Curves for thawing 4½-inch-thick blocks of frozen dressed cod in recirculated tap water at 45°F (7.2°C); water velocities were low and moderately low.

The curves in Fig. 3 present true gradients for solid-block thawing only up to about 1½ hr. After that time, several of the outside and top-of-block fish were separated and continued to thaw but at a faster rate. The latter were taken from the water upon reaching removal temperature. With one exception, the curves in Fig. 6 present true gradients for solid block thawing for the whole period of immersion. The time for the last fish to reach removal temperature in an undisturbed block was 7.7 hr. True gradients for block thawing of undisturbed fish in 45°F (7.2°C) water are shown by curves A, B, C, of Fig. 5.

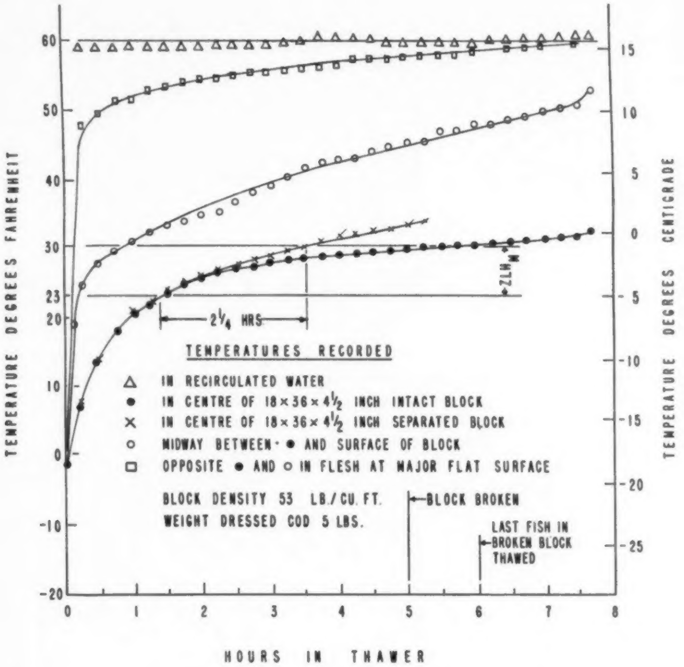

FIG. 6. Curves for thawing 18 × 36 × 4½-inch blocks of frozen cod in 60°F (15.5°C) tap water recirculated at a velocity of 4.0 ft/min (2×10^{-2} m/sec).

FACTORS AFFECTING THAWING TIME

Tables II and III and Fig. 7 and 8 give data obtained by thawing blocks of constant density (53 lb/ft^3 or 850 kg/m^3), length (36 inches or 914 mm) and thickness (4½ inches or 115 mm) and of various heights of 4–20 inches (102–508 mm). Water temperatures were 45 and 60°F (7.2 and 15.5°C). Water velocities were 0.8, 4 and 8 ft/min (4×10^{-3}, 2×10^{-2} and 4×10^{-2} m/sec), which as shown earlier did not contribute to different rates of thawing. A water velocity of 4 ft/min was used in a majority of runs at 45°F and in all at 60°F.

TABLE II. Experimentally-determined and average times to warm last-to-thaw block-frozen cod from 0°F (−18°C) to break-up state and to removal temperature while immersed in 45°F (7.2°C) recirculated water (block size: 4½ inches thick by 36 inches long; block height proportional to weight; water velocity low to moderately low).

	Time in hours					Time in hours			
	To warm fish sufficiently to permit breaking block		To warm last-to-thaw fish to removal temperature			For temperature in centre of undisturbed block to reach removal temperature		For last-to-thaw fish in undisturbed block to reach removal temperature	
Block weight	Experimental	Averages for regression curve (Fig. 7)	Experimental	Averages for regression curve (Fig. 7)	Block weight	Experimental	Averages for regression curve (Fig. 8)	Experimental	Averages for regression curve (Fig. 7, 8)
lb					*lb*				
30	3.4	3.2	4.4	4.5	30	6.0	5.9	8.0	7.9
30	3.2	3.2	4.7	4.5	30	6.0	5.9	8.0	7.9
30	3.2	3.2	4.8	4.5	30	6.5	5.9	7.7	7.9
45	3.4	3.6	4.8	5.0	45	5.5	6.4	8.0	8.2
45	3.4	3.6	4.8	5.0	45	6.0	6.4	8.0	8.2
75	4.0	4.3	5.2	6.0	72	8.0	7.2	9.0	8.7
75	4.3	4.3	6.7	6.0	75	7.2	7.3	9.0	8.7
95	4.0	4.7	5.5	6.6	86	7.2	7.7	9.2	8.9
96	5.5	4.8	7.3	6.6	88	8.0	7.7	9.5	9.0
99	5.0	4.9	7.2	6.7	95	–	–	8.0	9.1

Table III. Experimentally-determined and average times to warm last-to-thaw block-frozen cod from 0°F (−18°C) to break-up state and to removal temperature while immersed in 60°F recirculated water (block size: 4½ inches thick by 36 inches long; block height proportional to weight; water velocity low to moderately low).

	Time in hours			
	To warm fish sufficiently to permit breaking block		To warm last-to-thaw fish to removal temperature	
Block weight	Experimental	Averages for regression curve (Fig. 7)	Experimental	Averages for regression curve (Fig. 7)
lb				
20	2.0	2.0	2.5	2.7
27.5	2.2	2.3	3.2	3.0
32.5	2.4	2.4	3.6	3.2
35	3.0	2.5	3.8	3.3
53	2.5	3.0	3.6	4.0
56	2.9	3.1	4.1	4.2
61	3.8	3.3	4.8	4.4
88.5	3.3	4.1	4.8	5.5
95	5.0	4.3	6.0	5.8

	Time in hours			
	For temperature in centre of undisturbed block to reach removal temperature		For last-to-thaw fish in undisturbed block to reach removal temperature	
Block weight	Experimental	Averages for regression curve (Fig. 8)	Experimental	Averages for regression curve (Fig. 7, 8)
lb				
20	3.7	2.9	–	–
20	2.3	2.9	4.7	4.4
20	2.0	2.9	4.0	4.4
30	3.5	3.3	4.8	4.8
30	3.5	3.3	4.8	4.8
30	4.3	3.3	4.5	4.8
45	3.3	3.8	5.8	5.5
45	5.5	3.8	5.8	5.5
45	3.7	3.8	–	–
70	4.3	4.7	6.3	6.6
70	5.5	4.7	–	–
96	5.5	5.6	7.7	7.7

TEMPERATURE OF THAWING WATER

The top four regression curves[3] in Fig. 7 present the averages of experimentally determined points shown in Tables II and III. Weight or height of block is the independent variable. It will be seen that the last-to-thaw fish in broken blocks of 20-inch height thawed in 45°F water reached removal temperature about one hour later than those thawed at 60°F (15.5°C); the difference with fish in unbroken blocks was about 1½ hr. The less the height of blocks, the greater were the differences in time of thawing at the two temperatures. All fish in 95–100-lb (43–45-kg) blocks immersed in 60°F water and separated prior to complete thawing were ready for removal in under 6 hr. Those in 75-lb (34-kg) blocks of the same thickness, thawed at 60°F, reached removal temperature in less than 5 hr.

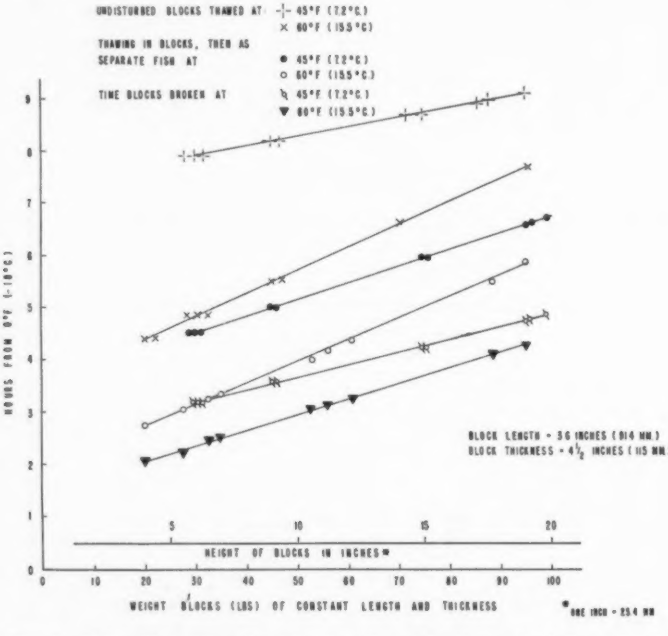

FIG. 7. Regression curves based on experimentally-determined times to raise last-to-thaw block-frozen dressed cod to the break-up state and to removal temperature and those in unbroken blocks to removal temperature. Initial temperature of fish 0°F (–18°C); temperature of water 45°F and 60°F (7.2°C and 15.5°C); water recirculated at low to moderately low velocities.

Unlike results with individual fish (Magnusson, 1955) the rate of thawing in block-frozen cod with and without separation of individuals did not increase in direct proportion to the difference between water temperature and 30°F. If actual temperature differences measured in blocks of undisturbed fish thawed at 60°F rather than at 45°F (thus doubling the temperature difference between water and 30°F) was the only influencing factor we should have experienced increased thawing rates of 65% (45-lb blocks), 55% (75-lb blocks) and 40% (100-lb blocks). These results were not obtained. Actual increases in thawing rate of last-to-thaw fish were 33, 22 and about 15%, respectively, for blocks of the three sizes. With doubled temperature differential, actual increases in thawing rate of last-to-thaw fish which had been separated from one another prior to completion of thawing were 26, 17 and 12%, respectively.

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[3] See Linear Regression in *Statistical Methods*, 5th Edition, by George W. Snedecor. Iowa State Univ. Press, Ames, Iowa, U.S.A.

BLOCK HEIGHT

Separated and unseparated fish in blocks of various heights did not thaw at a constant rate associated with conduction of heat to an infinite slab of uniform density (Fig. 7). Edge and other effects are believed to be responsible for the break-up state occurring sooner in blocks of decreased height than in those of full height.

As shown in Table III and Fig. 7, fish in 4½-inch-thick blocks of about 8-inch height reached removal temperature in recirculated water at 60°F in little more than 3 hr. This was about twice the time required to thaw fish of the same type pack in a commercially available dielectric fish thawer.

SEPARATION OF FISH

An important function of the curves in Fig. 7 is to show the benefit of separating the fish in the various sized blocks before all are raised to removal temperature. In the case of blocks immersed in 45° and 60°F water, from 2 to 3 and 1½ hr, respectively, were saved by separating the fish. At 60°F this saving was substantially independent of block height. It would be expected from theory that the time elapsed between separating the cod and their reaching removal temperature would be independent of block height. Experimental data give some support.

BLOCK THICKNESS

Only minimum information was sought on the effect of block thickness, principally because plate spacing of 4–4½ inches is needed in vertical plate freezers to provide sufficient room for packing. Moreover, a 4-inch-thick pack is required for some of the larger cod available. Comparative data on thawing times of 4- and 4½-inch-thick blocks in 60°F water recirculated at 4 ft/min may be obtained from Fig. 3 and 7. Times for blocks 14 inches (356 mm) in height were: (a) 4-inch-thick pack—complete separation of all cod in 2¾ hr, all fish reaching removal temperature in a total of 3¼ hr; (b) 4½-inch-thick pack—complete separation of all cod in 3½ hr, all fish reaching removal temperature in a total of 4½ hr.

ORIENTATION OF BLOCKS IN THAWER

A preliminary study of thawing times for blocks thawed on their ends, rather than on their long edges, was conducted. Five full-sized blocks about 18 × 36

× 4½ inches, each weighing about 100 lb, were submerged on their ends in water of 60°F and at a velocity of 0.8 ft/min. The sides of the thawer (Fig. 1) had to be extended to contain the extra depth of water required for the test. Two thawing runs were conducted. The fish in three blocks were separated part-way through the thawing process. Two unbroken blocks were thawed. There was no measurable difference in elapsed time to block break-up or in the total thawing time as compared with times obtained in previously described thawing runs (Table III).

USE OF SODIUM ALGINATE IN BLOCK-FROZEN COD

It was of interest to know what effect immersion, prior to plate freezing, in a solution having an initial freezing point of about 28°F[4] (–2°C) would have on elapsed time to fish break-up and to removal temperature. Accordingly, two 4½-inch-thick blocks of 4-lb dressed cod were frozen in an alginate solution. The cod were thawed in 60°F water recirculated at 4 ft/min. All fish in an 86-lb (39-kg) block were separated in 3¼ hr and had reached removal temperature in 4½ hr. Corresponding times for fish not packed in alginate were 4 and 5½ hr (Fig. 7). All fish in a 73-lb (33-kg) alginate-treated block which was not disturbed were ready for break-up and removal in 5½ hr. This time may be compared with a thawing time of 6¾ hr (Fig. 7) for conventionally frozen blocks, undisturbed during thawing.

There would be some advantage in packing cod in alginate to increase plant throughput but benefits in this regard would have to be weighed against the relatively high costs of alginates and the labour involved.

CONDITION IN UNDISTURBED BLOCKS

Considerable resistance to warming to removal temperature was shown by some fish in the interior of undisturbed blocks at the time when thermocouples indicated a temperature of 30°F (–1.1°C). This effect, shown in Tables II and III and Fig. 7 and 8, was found consistently. It would appear that two, three or four thermocouple readings at the centre of a block may give an unreliable and overly optimistic picture of the state of thawing. While thawing 88- to 96-lb (40- to 44-kg) blocks at 45°F for example, it was found that, on the average, a block could be broken at 4¼ hr but if it was undisturbed, it would retain its form for as long as 9½ hr (Table I) while exterior fish were at 45°F and a few ice pockets remained within the block, keeping a few fish at 30°F. Thus the block was held together. This points to the great advantage in breaking the blocks when interior fish are about 28–29°F (–2.2 to –1.7°C). The removal of a few fish by hand at an early stage is not justified from practical gains in thawing the remainder. Break-up with one effort (hand operation) or break-up at specific intervals in an automatic thawer would appear to be a practical procedure.

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[4] Formula 2 (Plain Kelgin) including salt and glycerine. The Kelco Co., 120 Broadway, New York 5, N.Y. Canadian Sales Agents: Charles Tennant and Co., (Canada) Ltd., Room 440, 6555 Cote Des Neiges Rd., Montreal 26.

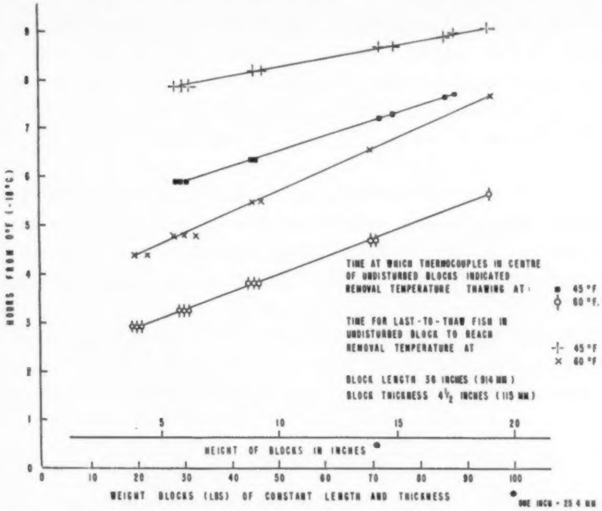

FIG. 8. Regression curves based on experimentally-determined times to raise last-to-thaw dressed cod in unbroken blocks from 0°F (–18°C) to removal temperature and on thermocouple readings in the centre of the blocks. The blocks were thawed in water at 45°F and 60°F (7.2°C and 15.5°C) recirculated at low to moderately low velocities.

USE OF CURVES

The curves (Fig. 7 and 8) can be used to predict thawing times and to select block height to realize preselected thawing times in 45 or 60°F water. They show times at which partly thawed blocks can be broken, the relation of these times to total thawing time and to the time undisturbed blocks remain intact.

DISCUSSION

In commercial practice, temperature differences (averaged over the whole of the freezing plate area) producing the driving force for removing heat from fish by conduction can be about the same as those for adding heat from 60°F (15.5°C) water during the thawing operation (Fig. 2 and 3). Thus, equal thicknesses of block-packed fish should be frozen considerably more quickly than they can be thawed in water as the frozen material has a conductivity about three times that of the unfrozen material (Langstroth, 1931; Long, 1955). This is true for solid blocks of fillets, but not necessarily true for blocks of unfilleted cod (Fig. 2 and 3). The latter are packed with sufficient voids to enable the individual fish to be separated at an intermediate stage in the thawing operation and this speeds thawing. Blocks with uneven surfaces provide only partial contact of fish and freezer plate but the area of contact of the fish with a water bath will almost always be greater than the area of the block projected. While thawing conditions in the full-sized block approach those in an infinite slab, and thawing proceeds principally in a direction perpendicular to the major surfaces, conditions in a block of somewhat less than full height are influenced significantly by transfer of heat at all surfaces and blocks of reduced height thaw considerably faster than those of full height. While part-sized blocks can be thawed conveniently, they cannot be frozen as economically or as conveniently as full-sized ones. The net effect is a relative increase in thawing as compared to freezing rate to the extent that blocks of about 15 inches in height have comparable freezing and thawing times.

The carry over to the commercial stage of the main principles of successful water thawing (e.g. observance of suitable water recirculation rates and temperatures) do not pose particular problems. But blocks must be broken for maximum thawer throughput. In British-designed air thawers (British Patent Application No. 22121) cod in blocks fall apart at various stages of thawing with the application of slight gravitational forces. It would appear to be desirable to create approximately equal block-disrupting forces in the large scale water thawer operating on either the batch or the semi-continuous delivery principle.

SUMMARY

Thawing time in water for rectangular-shaped blocks of frozen dressed cod was investigated. Thawing rates were not changed by using fish of 2–6 lb (1–3 kg) dressed weight as long as blocks of fairly uniformly sized fish were of equal weight and thickness. Larger fish could be separated sooner from one another than could smaller individuals but took longer to thaw after separation. Similar rates of thawing were achieved at low and moderately low water velocities of 0.8, 4 and 8 ft/min (4×10^{-3}, 2×10^{-2} and 4×10^{-2} m/sec). Separation of fish from the blocks when the coldest fish were at a temperature of about 28–29°F (–2.2 to –1.7°C) materially reduced thawing time. Sodium alginate used in the blocks during packing prior to freezing permitted still earlier break-up of the frozen fish. Undipped fish in blocks of 4½-inch (115-mm) thickness weighing 75 lb (34 kg) were separated and thawed in 60°F (15.5°C) water in 4½ hr, this being about the time for freezing between plates under commercial conditions. Blocks, weighing 35 lb (16 kg), of the same thickness were thawed in 60°F water in about 3 hr or in twice the time taken in a commercially available dielectric thawer. Thawing blocks in recirculated water thus merits consideration with other methods. Thawing in 60°F (15.5°C) versus 45°F (7.2°C) water improved the thawing rate by 12 to 26% for broken blocks, depending upon block height. Other factors being constant, reduction in block height improved the thawing rate, probably as the result of edge effects. Reduction in block thickness also improves the thawing rate. Densely packed (58 lb/ft^3) 4-inch-thick blocks weighing about 80 lb were thawed in 60°F water in about 3 hr. For the Newfoundland area, block thickness cannot be reduced much below 4 inches (102 mm).

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Activity Cycles in the Brown Trout (*Salmo trutta* L.)
2. Fish Artificially Fed[1]

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ABSTRACT

The diurnal and annual locomotory activity cycles of four brown trout confined in separate cages on the bed of Windermere and fed artificially from the lake surface are described. The annual activity curves were very similar, the fish being less active during the winter than in the summer with maximum activity occurring in June and again in August. The diurnal activity patterns were also very similar with a sharp rise in activity at dawn each day after an inactive night, the increased activity being maintained throughout the light period and falling rapidly at dusk.

Two of the fish were fed daily at irregular times and two were fed automatically every 2 hr. The similarity of the activity patterns of these four fish and also the similarity to the patterns of unfed fish described in a previous paper suggests that light and not food is the prime stimulus to locomotory activity in brown trout.

INTRODUCTION

In a previous paper (Swift, 1962) the activity cycles of brown trout confined in netting cages on the bed of Windermere were described. These fish, while living under these conditions, and completely dependent on the food which reached them through the netting of the cage, exhibited a definite annual and diurnal cycle of locomotory activity. However the cause of these activity cycles was not apparent from this work, or from observations of fish activity cycles by previous workers (Alabaster and Robertson, 1961; Hirata, 1957; Hirata and Kobayashi, 1956; Kuchin, 1900; Musnic, 1931; Oliphan, 1951; and Sushkino, 1939). All these workers, with the exception of Alabaster and Robertson, associated the diurnal activity cycle with a feeding cycle; Alabaster and Robertson recorded a similar activity cycle in fish which were artificially fed. In the previous experiment the fish had been deliberately kept under conditions which resulted in them being underfed and in poor condition. This work was repeated with new fish which were well fed. Two were fed once a day and two others were fed automatically every 2 hr, in order to compare the activity cycles of underfed fish, fish fed once a day and fish in continuous contact with abundant food.

METHODS

The apparatus used in this experiment has been previously described (Moore, 1961; Swift, 1962). The fish were confined in netting cages 8 ft (2.4 m) long with

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a 2-ft (0.6-m) cross section; these cages were sunk in 6–8 ft (1.8–2.4 m) of water and raised 2 ft (0.6 m) from the bed of the lake on wooden legs. Four identical cages were used and in each cage was placed one hatchery trout between 20 and 26 cm long. Hatchery fish were used for this work as these more readily take the liver used to feed the fish during this experiment. The fish were fed with minced beef liver set in gelatine (Swift, 1960) passed down a tube from the water surface. Fish 5 and 6 were fed at irregular times every day except Sunday. Fish 3 and 4 were fed every 2 hr (night and day) from an automatic feeding device started on May 11. The experiment started on January 20 and finished on October 31, 1961. The passage of a fish between three pairs of electrodes evenly spaced down the length of the cage was recorded by a pen as a mark on a moving chart. These marks were counted to give the hourly and weekly activity totals. A fifth cage was kept empty to maintain a check on the functioning of the recording apparatus. The water temperature was taken daily with a thermistor, and total solar radiation was recorded with a moll-thermopile. At the end of the experiment the fish, which were in excellent "condition", had increased in length an average of 30.7%.

RESULTS

The diurnal and annual changes in the fish's locomotory activity are shown in Fig. 1. The monthly means of the total number of times a fish passed between a pair of electrodes in its cage in an hour have been plotted to show the diurnal variation in activity. These hourly totals have been summed to give the weekly average of the total number of passages during each day (Fig. 2). The weekly average water temperature and the ten-day average total solar radiation on a horizontal plane near the lake surface are shown (Fig. 2).

The annual activity curves for the fish show an overall similarity; after a low level during the early part of the year the activity increased during May to reach a maximum in June, falling again during July and rising again in August in all the fish with the exception of fish 6. At the end of the experiment it was found that fish 6 was the only female in the group and the indications were that, in common with the other fish it had been ripe during the autumn. It is considered possible that this rise in activity during the autumn was in fact connected with the fish's sexual maturation. There is no detailed similarity in the curves of the annual activity of the four fish.

The diurnal activity patterns for all four fish were very similar throughout the year (Fig. 1). During January, February and March there appeared to be only a random variation in the activity of the fish over the 24 hr of the day. In March, fish 3 began to show the diurnal rhythm subsequently shown by all the fish for the rest of the experiment. The most obvious feature of this rhythm was the marked rise in activity which occurred at dawn; this increased activity was maintained throughout the hours of daylight and fell at dusk each day. The changes which occur in the level of activity at different times of the year are caused by a change in the level of activity maintained by the fish during the hours of daylight and also by the length of time that this activity is maintained.

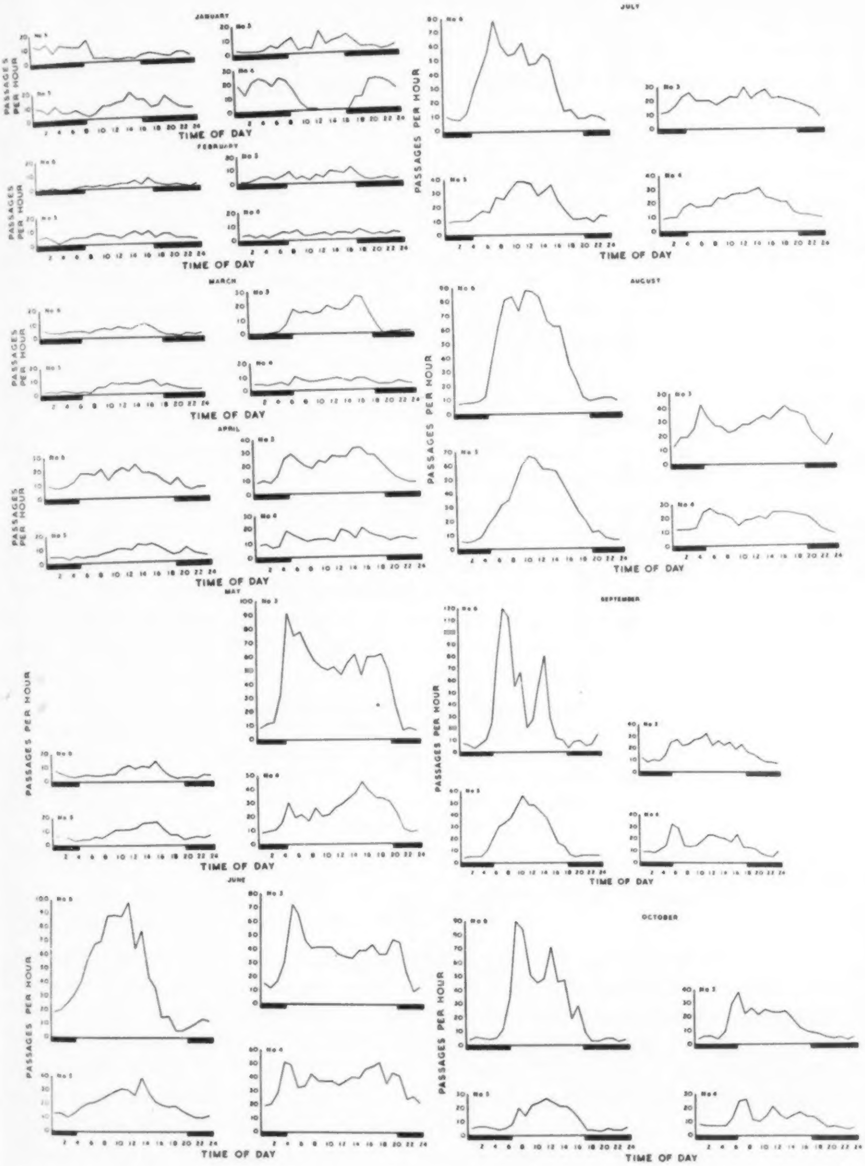

FIG. 1. Locomotory activity of brown trout recorded as monthly average of the total passages between fixed electrodes during each hour of the day. The thick black part of the abscissa indicates the hours of darkness. Fish 3 and 4 fed every 2 hr; fish 5 and 6 once daily.

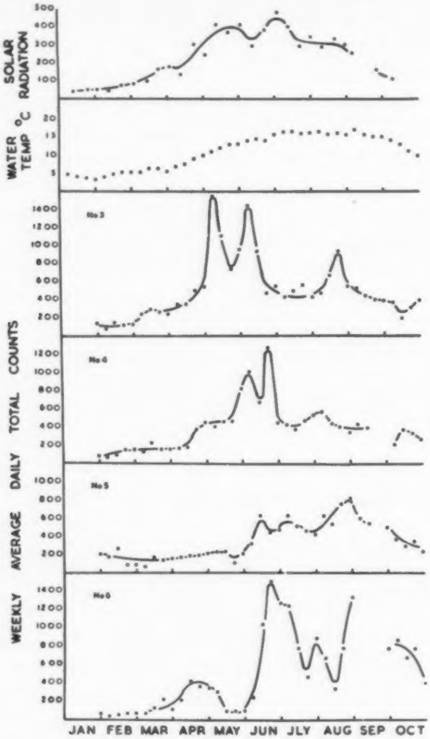

FIG. 2. The weekly average of the total locomotory activity of brown trout in relation to solar radiation and water temperature.

Seasonal changes also occur in the level of activity at night, but only to a much smaller extent.

DISCUSSION

In the previous paper of this investigation (Swift, 1962) the activity cycles of underfed trout completely dependent for their food on the haphazard supply of invertebrates reaching them through the netting walls of their cages were shown. In this report the activity cycles of well-fed fish either fed once a day or in continuous contact with food are shown. There is no great difference between either the diurnal or the annual changes in the activity of these fish living under three different feeding regimes. Particularly similar are the diurnal activity patterns of all these fish and also the seasonal changes in these diurnal activity patterns. The commencement of this feeding rhythm did not in any noticeable way alter the activity pattern of the fish. Unlike the other fish, fish 4 did show a large increase in activity during May, but this increase began before the automatic feeder was operating and therefore cannot be attributed to the 2-hr feeding. It is worth emphasising that the 2-hr feeding was not in any way reflected in the diurnal activity pattern of the fish. These results suggest that it is not the sight or smell of food which is the prime stimulus for locomotory activity in brown trout; in fact the opposite would seem to be the case. The trout feeds because it is active, and is not active because it is feeding.

A diurnal activity pattern such as exhibited by these trout seems to be common to many species of fish. The following workers all observed a similar rhythm in activity with a marked increase at dawn in the following species of fish: Alabaster and Robertson (1961), in roach (*Rutilus rutilus*), bream (*Abramis brama*) and perch (*Perca fluviatilis*); Hirata (1957) and Hirata and Kobayashi (1956), in goldfish (*Carrassius auratus*); Oliphan (1951), in Baikal grayling (*Thymallus articus baicalensis*); Oliphan reports similar observations by Kuchin (1900), in roach (*Rutilus rutilus*), bleak (*Albernus albernus*), Prussian carp (*Carassius auratus gibalio*), tench (*Tinca tinca*), perch (*Perca fluviatilis*), ruff (*Acerna cernua*), and burbot (*Lota vulgaris*); Musinic (1931) and Sushkino (1939), in herring (*Clupea harengus*). Thus many different species of fish living under widely different conditions all exhibit a similar diurnal rhythm in their locomotory activity. Since the one common variable in all these situations is the variation in the day length, it would seem that it is the light which stimulates these fish to become active. It was thought that perhaps when it was light the fish were able to see their food and thus became active, but in this experiment those fish which were fed once a day were as active at dawn as the fish receiving food every 2 hr. The cause of the seasonal variation in locomotory activity is in no way obvious. Why, for instance, the activity should fall during July remains a mystery. There is no obvious correlation between the seasonal variations in locomotory activity of the fish, the water temperature, or the amount of solar radiation incident on the water surface.

ACKNOWLEDGMENTS

I wish to acknowledge my debt to W. H. Moore, who designed, built and maintained the apparatus. My thanks are due to H. C. Gilson for a critical reading of the manuscript.

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The Cell Division Rates of Some Marine Phytoplankters as a Function of Light and Temperature[1]

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ABSTRACT

An apparatus is described for exposing phytoplankton cultures to 64 different combinations of light and temperature. The light was of a known spectral energy resembling sunlight after passage through a few metres of clear coastal sea water and was measured in the energy units of cals/cm^2/min (langlies/min or ly/min). The temperature range used varied in each experiment, the range of illumination was from about 0.005 to 0.4 ly/min, the latter being equivalent to bright summer sunlight at a depth of only a few metres in the sea.

The growth of five bacteria-free cultures, *Dunaliella tertiolecta, Amphidinium carteri, Monochrysis lutheri, Skeletonema costatum* and *Thalassiosira nordenskiöldii* was followed for a period of 3–5 days using a cycle of 16 hr illumination and 8 hr of darkness every 24 hr. Growth was measured by counting cell numbers and the rate constants for cell division were evaluated at standard times.

The resulting growth-response curves are reported and discussed in relation to their ecological significance. Except for *T. nordenskiöldii* cell division was relatively insensitive to temperature over a range exceeding 10°C. Cell division showed a "stress" response, the growth rate being more susceptible to extremes of illumination when the cells were near to the extremes of temperature for a given species and to extremes of both temperature and light if the chemical nature of the medium was unsatisfactory. At optimum temperatures there was no inhibition of cell division at intensities of 0.4 ly/min of photosynthetically active light.

INTRODUCTION

It is customary to measure phytoplankton growth in terms of photosynthesis (oxygen evolution or carbon-14 uptake) but cell division kinetics, especially of marine phytoplankters, have received comparatively little study (Braarud, 1937, 1944; Stanbury, 1931; Spencer, 1954; Sweeney and Hastings, 1958; Kain and Fogg, 1958 a, b, 1960; Curl and McLeod, 1961; Lanskaya, 1961; Mjaaland, 1956). Cell division rates, *per se*, are of considerable interest, however, as the cell division of plants does not necessarily respond to changes in growth conditions in the same manner as photosynthesis. This has been demonstrated in the case of *Chlorella* by Sorokin (1960). If cell division and photosynthesis take place at different speeds, cells must either change in size (Myers, 1946, Winokur, 1948, 1949) or secrete organic matter. Both phenomena could take place simultaneously.

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In nature there are ecologically significant differences between cell growth and cell multiplication. A given plant biomass in the form of 2 cells may be less susceptible to loss by predation than if present in only one cell.

Phytoplankton growth responds in a complex manner to the combined effects of temperature, light, salinity and nutrition. In the present study of cell division only the effects of the first 2 variables have been investigated, using sea water of about optimum salinity and what is thought to be near optimum chemical composition. The problem of examining the effects of a large number of combinations of light and temperature simultaneously has been overcome by using equipment having a cross gradient of the 2 variables (cf. Halldal and French, 1958).

The present work describes the design and use of a new apparatus for determining the kinetics of cell division under controlled and measurable conditions of illumination and temperature, and illustrates its use with 5 species of marine phytoplankters.

EXPERIMENTAL METHODS AND OBSERVATIONS

Cultures and Media

All phytoplankters were from bacteria-free cultures of the following origin:

Dunaliella tertiolecta. Woods Hole Oceanographic Institution. Clone "Dun". Origin not known.

Amphidinium carteri. Woods Hole Oceanographic Institution. Clone unknown but isolated either from a rock pool at Woods Hole or from Falmouth Great Pond.

Monochrysis lutheri. Haskins Laboratory, New York.

Skeletonema costatum. Woods Hole Oceanographic Institution. Clone "Skel". Isolated from Long Island Sound.

Thalassiosira nordenskiöldii. Narragansett Laboratory, University of Rhode Island, isolated from Narragansett Bay.

For experiments with the three flagellates a synthetic sea water was used containing analytical reagent quality $NaCl$; $MgCl_2.6H_2O$; Na_2SO_4; KCl; and $CaCl_2.2H_2O$ to give a solution with the major ions present in the same proportions as in sea water with a salinity of about 29%. The solution was neutralized to pH 7 with $NaOH$; sodium bicarbonate and boric acid were added to give twice the amount found in sea water of this salinity. For experiments with the diatoms, surface water of a salinity about 32‰ was taken from the surface of the northeast Pacific Ocean at 50°N, 145°W, about 500 miles from the coast. This water, aged several months and filtered through a membrane filter before use, was found to support a vigorous growth of diatoms, being much more satisfactory for this purpose than synthetic sea water and better even than some coastal sea water samples.

Both synthetic and natural sea water samples were enriched with trace metals by the addition of 1 ml/litre of the following solution: 1000 mg $FeCl_3.6H_2O$; 620 mg $MnSO_4.H_2O$; 250 mg $ZnSO_4.7H_2O$; 130 mg $Na_2MoO_4.2H_2O$; 40 mg $CoCl_2$.

TABLE I. Summary of light and temperature effects.

Species	Range of cell concentrations (cells/l.$\times 10^{-b}$)	Preconditioning temperature(°C)	Optimum growth conditions[b]		Maximum rate[c] $k_{max} \times 10^{3}$	*k.* max		Ranges for: $0.66k_{max}-1.0k_{max}$		Any growth[a]	
			°C	ly/min		°C	ly/min	°C	ly/min	°C	ly/min
Dunaliella tertiolecta	40–1500	18	25	0.25	*30* Some initial values of *38* at 10 hr	28–33	0.15–0.4+	19–36	0.15–0.4+	11–36+	0.02–0.4+
Amphidinium carteri	20–250	23	24	0.30	*12* No initial high value	23–26	0.1–0.4+	21–32	0.050–0.3	18–33	0.05–0.4+
Monochrysis lutheri	150–2000	18	19	0.15	*15* Some initial values of *16.7*	17–24	0.1–0.2	14–24	0.075–0.30	8–27+	0.015–0.6+
Skeletonema costatum	30–1000	18	20	0.25	*30* No initial high values but maximum rate over a wider temperature range at *10* hr	16–26	0.075–.04+	15–28	0.13–0.4+	6–28+ (30° max. ?)	0.005–0.4+
Thalassiosira nordenskiöldii	10–70	10	13	0.075	*20* No initial high values	11.5–14	0.02–0.15	9.5–15.5	0.02–0.2	less than 2°C to 19°C	0.005–0.5+

[a] No cell division outside these ranges but not necessarily at all points within.

[b] Values selected for high growth rate and least susceptibility to changes of light and temperature.

[c] Maximum steady rate.

$6H_2O$; 40 mg $CuSO_4.5H_2O$ and 6 g of disodium ethylenediaminetetraacetate made to 1000 ml and adjusted to pH 7.5.

Growth factor additions were made with 1 ml/litre of the following solution: 5000 mg inositol; 3000 mg thymine; 500 mg thiamine hydrochloride; 100 mg nicotinic acid; 100 mg calcium pantothenate; 100 mg p-aminobenzoic acid; 20 mg folic acid; 10 mg biotin; and 2 mg vitamin B_{12}—all dissolved in 1000 ml of water.

Solutions for use with the flagellates were buffered with 5 ml/litre of 20% "Tris" solution at pH 8 but this was omitted from the media used for the diatoms in case it had an adverse effect on their growth.

All media were enriched with 500 μg-at/litre of nitrogen as potassium nitrate, 50 μg-at/litre of phosphorus at K_2HPO_4. Media for the diatom experiments were also enriched with 500 μg-at/litre of silicon as neutralized sodium silicate. Experience had indicated that these concentrations had no significant effect on growth kinetics and that nitrogen became the limiting nutrient after excessive phytoplankton growth.

Glassware and media were steam-sterilized at 15 psi for 20 min. Phosphate was sterilized separately and added aseptically to the media when no Tris buffer was present and the cool solutions were aerated vigorously with filtered air to reduce the pH to about 8.0.

Five hundred millilitres of media were inoculated with a suitable small volume of bacteria-free phytoplankter culture and grown for 2–3 days at the preconditioning temperature (Table I) under cool-white fluorescent light of about 0.065 ly/min intensity. Experiments were started in the late afternoon on the day when cell concentrations had reached the desired level. The flask was darkened a few hours before the experiment commenced to initiate the light-dark cycle used in the main experiment.

LIGHT SOURCE AND MEASUREMENT

The problem of getting an intense illumination with a spectral distribution not unlike that of sunlight in sea water was solved by using tungsten light filtered through a chemical solution. High intensity sources are available from discharge tubes but they are either inconvenient in shape or have an undesirable fraction of the total radiant energy at fixed wavelengths. A more sophisticated filter system than the one used here could have been devised by using several layers of coloured glass but it is doubtful if it would have merited the high cost and the serious attenuation of radiant energy inherent in such systems.

The lamps used were of the high temperature tungsten filament type, in quartz envelopes containing iodine vapour to prolong the lamp-life to 2000 hr. Direct measurement with an optical pyrometer gave a filament temperature of 3000°K at the operating voltage. Figure 1 shows the relative emission of the lamp together with the transmission characteristics of the chemical filter, which was 2.0 cm of a solution containing 4.0 g $CuSO_4.5H_2O$; 20 g $(NH_4)_2SO_4.FeSO_4.6H_2O$ dissolved in 100 ml of *air-free* water containing 5 ml of syrupy phosphoric acid. (The phosphoric acid prevents light absorption in the visible and near ultra-violet by traces of ferric ion but the solution must be oxygen-free or a white ferric phosphate complex will slowly precipitate.) The spectral distribution of the resulting filter tungsten light is given in Fig. 2 together with the emission spectra of "cool white" and "daylight" fluorescent lamps.

Intensities were measured by a specially designed Weston photocell which recorded intensities at various depths in the tubes used for holding cultures. The photocell was calibrated under the filtered tungsten light source against a radiometer (Stephens and Strickland, 1962) to enable illumination to be expressed as cals/cm^2/min (langlies/minute, referred to as ly/min in the text).

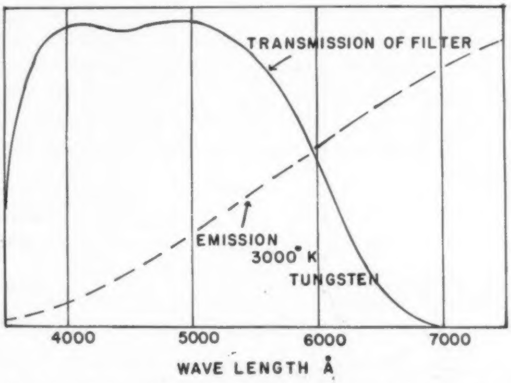


FIG. 1. Spectral characteristics of tungsten light and filter.

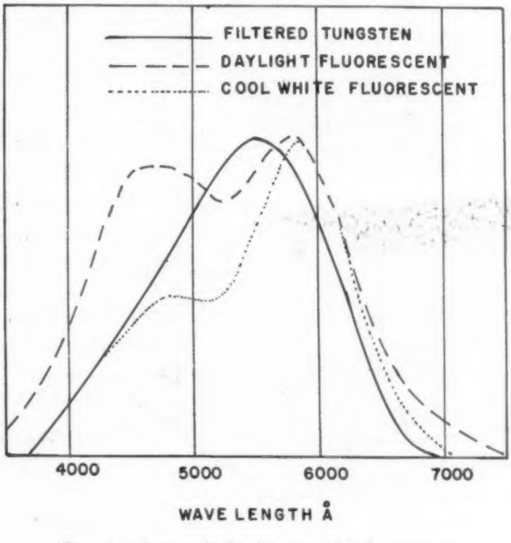


FIG. 2. Spectral distribution of light sources.

An illumination of over 0.5 ly/min could be obtained although a maximum of only about 0.4 ly/min was used in most of the experiments. This intensity is still over three times the intensity of phytosynthetically active light that can be obtained from fluorescent lighting.

THE APPARATUS

The equipment was developed and modified throughout the work, without affecting the light-temperature characteristics. The final design is shown in Fig. 3. Light from two 1500-watt General Electric T3/Q lamps in special parabolic reflectors was focussed to give the minimum divergence at the level of the aluminum block. The lamp voltage was controlled manually to be constant between 280 and 300 volts according to the age of the lamps. Light passed through 7 cm of rapidly flowing water which removed most of the heat and then through a 2-cm "sandwich" of Perspex containing the chemical filter. The barrier between the top of the chemical filter and the cooling-water was made of thin glass to facilitate a conduction of heat from the chemical filter into the cooling-water.

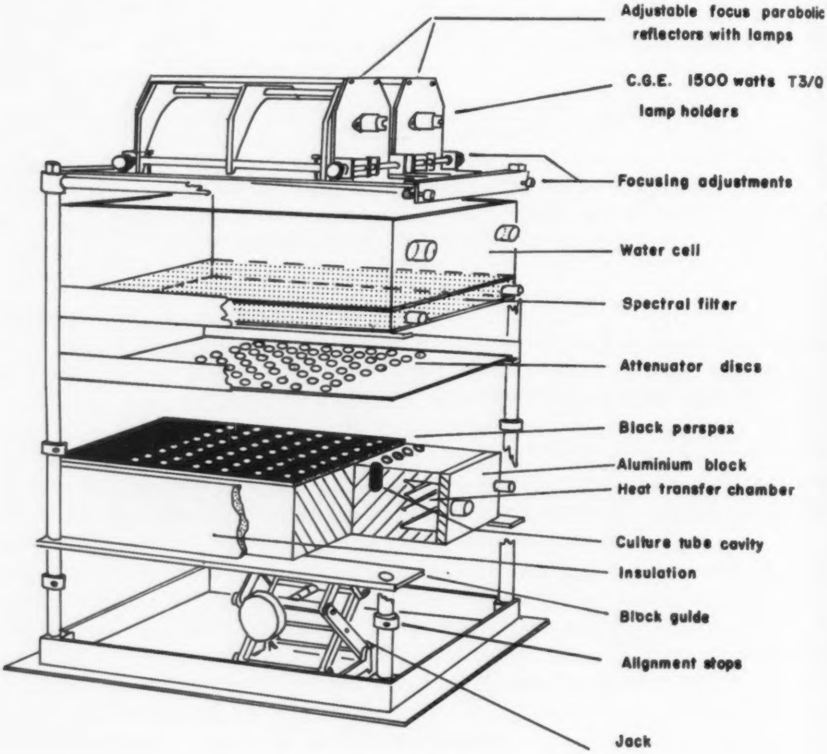

Adjustable focus parabolic reflectors with lamps
C.G.E. 1500 watts T3/Q lamp holders
Focusing adjustments
Water cell
Spectral filter
Attenuator discs
Black perspex
Aluminium block
Heat transfer chamber
Culture tube cavity
Insulation
Block guide
Alignment stops
Jack

FIG. 3. Diagram of apparatus.

The light was then attenuated by a set of fogged-film discs set into holders in an aluminum plate. Two or three attenuator discs could be stacked in each holder. It was possible to compensate for variations of light intensity along the length of the aluminum plate by a suitable selection of film densities, thus giving the same intensity of transmitted light through each of the 8 holes in a row parallel to the lamp axis. The intensity from row to row was varied from 0.4 ly/min to 0.01 ly/min in 8 steps, roughly in geometrical progression. (After seven days of continuous use the light-field changed by a maximum of 20% in some rows but generally by much less.)

The light illuminated small tubes containing the algal culture. The tubes were set into black-painted holes in an aluminum block, the 5 mm of tubes projecting above the block being surrounded by a black Perspex plate. The block was lowered and the black Perspex plate removed when samples were taken. During an experiment the plate was replaced and the block jacked up to give contact between the Perspex and the attenuator discs. The tubes were calibrated to hold 3.5 ml of culture in a column 20 mm deep and 16 mm in diameter. The collimation of light was such that the intensity of illumination at the base of a tube was generally greater than 75% of the illumination at the surface of the culture solution. However, the fogged-film attenuators introduced scatter with the result that only about 55–65% of the surface light reached the base of some tubes. All radiant energy values reported here are for the surface of the culture. The mean illumination of flagellate cells, which remained in suspension or were phototactic, was not far from the stated figures. The mean illumination diatom cells, which mostly settled during a 24-hr period, was probably some 25% less than the recorded values.

A temperature gradient was impressed on the aluminum block at right angles to the light gradient by circulating hot water through one chamber and a refrigerated methanol–water mixture through the chamber at the other end of the block. A heated bath and a refrigerated bath, both thermostatically controlled and of about 1 kw capacity each were used. An 18°–20°C temperature differential could be impressed over the 8 rows of culture tubes, the gradient being linear and uniform. This temperature range was found adequate for most experiments except when using the *Dunaliella* culture. With this organism the initial experiment had to be repeated using a higher temperature range. Temperatures were monitored continuously by thermistors set into culture tubes held in a central unlighted row. The temperature difference of about 2.2–2.5°C between each row of tubes could be controlled to better than 0.2°C an average being taken for the duration of an experiment.

EXPERIMENTAL PROCEDURE AND CELL COUNTING

All 64 tubes were filled from the same stock culture and the illumination turned on overnight for 16 hr. The lights were turned off during the day, which facilitated sampling and gave a light–dark cycle of 16 and 8 hr, respectively. Although in no sense ideal some such light–dark period was thought to be more realistic than continuous illumination. The cell division rate of a species and the interrelated effects of light and temperature doubtless depend on the light–

dark cycle (Tamiya *et al.*, 1955) but was not examined in detail. The minimum division times found in the present work were longer than those recorded in other experiments where there was continuous illumination. The increases (at optimum temperatures) varied between 25% (*Dunaliella*) and 40% (*Skeletonema*). If the increases had been directly proportional to the dark period they would have amounted to 33%.

Initially the tubes and cultures were bacteria-free but it was impossible to work the equipment under completely aseptic conditions. Care was taken to minimize contamination and remarkably little occurred, even after a week of operation. If the media and cultures were not initially bacteria-free, turbidities due to bacteria developed within two days.

Each morning, tubes were removed from the block, a few at a time, and held in a darkened container. They were refilled to the graduation with a few drops of sterile distilled water if necessary to allow for evaporation, which was appreciable in tubes held at temperatures much in excess of 25°C. The tubes were covered with pieces of sterilized Saran Wrap, shaken well to disperse the cells, and a drop removed for counting, using flame-sterilized Pasteur pipettes. Any excess cultures were returned to the tubes and they were replaced in the aluminum blocks with a minimum delay. Care was taken to prevent stray light entering the tubes in the block when this was lowered down from the attenuator plate.

Counting presented a considerable problem. Sample volumes were too small for a Coulter or other electronic counter to be used. The daily sampling load was much too high for direct microscopy. The problem was overcome by photographing the cells from each tube every day and examining the photographs later at leisure. Living cells were photographed directly in a hemocytometer, movement of flagellates being "stopped" by the camera at 1/50–1/100 second exposure. Duplicate photographs were taken, one of each grid of the hemocytometer, at a magnification of 20 × on 35-mm Kodak Plus X film. The total number of cells seen in both photographs exceeded 70 at the beginning of an experiment. Counts exceeding 300 were made where possible later in the run. The lowest cell concentration that could thus be measured directly with a 95% confidence limit of ±10% was about 50.10^6 cells/litre. By photographing replicates from the initial culture the minimum concentration could be reduced.

For larger cells (exceeding about $1000\mu^3$, e.g. the *Thalassiosira nordenskiöldii*) the maximum permissible initial numbers are too small for a hemocytometer to be used and the settling block shown in Fig. 4 must be employed. The counting pits were drilled through a Perspex block and were 6 mm deep and 4 mm in diameter. They were filled from Pasteur pipettes, one pit for each sample, and the block was exposed for a few moments to formalin vapour in an enclosed space to kill the cells. A coverslip was then placed over the block, excess liquid being drained away to leave 0.075 ml of fluid in each pit. Cells were allowed to settle for about 4 hr and the bottom of each hole photographed at a magnification of 20 × to give a rectangular field covering most of the area. Settling was generally uniform but, if not, the area photographed was selected to give as good

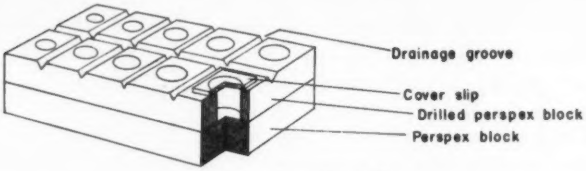


FIG. 4. Counting cell.

an estimate of the average number as possible. The number in each photographic field was related to the concentration by a factor determined from calibration against a hemocytometer. At least 100 cells were photographed at a beginning of an experiment corresponding to a cell concentration of about 5.10^6 cells/litre.

The 35-mm negatives were projected for counting. The hemocytometer grid was used as a counting reference or, where the settling pits were used, the negative was projected to cover a ruled grid on a standard screen.

CALCULATION OF GROWTH CONSTANT

Cell counts for each tube each day were plotted on semi-log paper and the best curve through the points drawn free-hand. During the exponential phase of growth straight line relationships were found, but this was often delayed due to periods of induction and adjustment. The slope of each curve was measured at the arbitrary standard times of 10, 32, 56, 80 and 104 hr, or as many of these as was appropriate for an experiment. From these slopes a growth constant in the expression, $\log_{10}n_2/n_1 = k\ (t_2\text{–}t_1)$ could be calculated where n_1 and n_2 were the number of cells at times t_1 and t_2, respectively. The growth constants for cell division in each tube at the standard times were plotted to give the three-dimensional response diagrams shown in Fig. 6–10.

Cell growth eventually slowed down or stopped for two reasons, depletion of nitrate or carbonate (the latter producing an undesirably high pH) or from a genuine reduction of growth rate due to the adverse effects of temperature and light. The maximum cell number that could be tolerated in each experiment before nutrient depletion interfered with growth was judged from the cell concentrations at the end of the exponential stage in the light and temperature region of best growth. For safety, no account was taken of rates unless cell numbers were less than about 75% of these values. The range of cell concentrations over which rate measurements were made is shown in the first column of Table I.

The regularity of cell division during each period of 24 hr was checked by only a few tests but the results of these and shape of the growth curves indicated that in most cases it was fairly uniform and continued unchanged for much of the 8-hr dark period (Sweeney and Hastings, 1958; Sorokin and Krauss, 1959). This "overshoot" of cell division which occurred in the dark after a period of illumination gave spuriously high division rates at the low light intensities for the first 12–24 hr after the solutions, precultured at 0.065 ly/min, were placed in the apparatus. This is well marked in the 10-hr rate graphs of *Dunaliella* and *Monochrysis* shown in Fig. 6 and 8.

TABLE II. Summary of growth behaviour.

Species	Minimum light for cell division		Temperature adjustments	Stress patterns (see text)	Other remarks
	ly/min	Range °C			
Dunaliella tertiolecta	0.015–0.025	22–34	General improvement below 18°C and extension of lower limit from 16° to 12°C.	Slight indication at highest measured temperature with both high and low light intensities.	Motility lost at temperature greater than about 25°C at light intensities less than about 0.05 ly/min although cell division still occurred. Below 25°C cells motile at low light intensities for 100 hr even although no cell division took place.
Amphidinium carteri	0.01–0.05 (erratic behaviour)	21–28	Extension of lower limit from 21°C to 18°C. Rapid growth ($k = 20 \times 10^{-3}$) where no growth for 2–3 days. See text.	At high temperatures with low light intensity. Not apparent at high temperature at 0.4 ly/min. No low temperature effects noted but delayed growth areas develop (see text).	Motility lost only if cells had stopped dividing.
Monochrysis lutheri	0.01–0.02	17–24	General improvement below 15°C and extension of lower limit from 11°C to 8°C.	At high temperature with high and low light intensity. Slight indication at low temperature with high light intensity. "Chemical" poisoning (see text) stopped growth except at 18–21° at about 0.1 ly/min.	Motility lost only if cells had stopped dividing.
Skeletonema costatum	0.003–0.006	16–28	Very marked improvement below 10°C most rapid at low light. Extension of lower limit from 10°C to 6°C.	No high temperature effects noted but found at low temperature with low light intensity. Not apparent at low temperatures at 0.4 ly/min. Low temperature adjustment less rapid at higher light intensities (see previous column).	Marked cell size changes. Small cells at low light and high temperatures. Large cells at high light and low temperatures. Division rates often similar under both sets of conditions.
Thalassiosira nordenskiöldii	0.005–0.01	5–15	No obvious changes with time.	Very well marked at high temperatures with both high and low light intensities. Very well marked at low temperatures with high light intensities. "Chemical" poisoning (see text) stopped growth except near 12°C at 0.02–0.08 ly/min.	No obvious change in cell size.

The division rates at 10 hr were also anomalous in the case of *Dunaliella* and *Monochrysis* because of the appearance of areas of extra rapid growth which did not show at 32 hr and later. The behaviour was most marked with *Dunaliella* at temperatures that were 5–15°C above the preconditioning temperature of the culture. One may speculate that in cases like this, when cells are subjected to a sudden increase of temperature from below optimum into the optimum temperature range of the species, cell division is accelerated temporarily before the enzyme systems have time to adjust to give a lower Q_{10}.

Finally, rates at 10 hr were unrepresentative because the growth at temperatures several degrees below the preconditioning temperature was abnormally slow. This was probably a result of the same effect that produced the extra fast growth, the enzymes systems again requiring time to adjust to give a new Q_{10}. Adjustments to a decrease in temperature, however, took longer and generally 48 hr or more was required for a steady state to be reached (Table II). For these reasons the characteristic cell division pattern for each species should not be taken from the 10-hr or 32-hr graphs. Data for Tables I and II have been obtained from 56-hr or 80-hr response diagrams, it being assumed that growth at 80 hr in the fast growing areas continued to be the same as the 56 hr, even although such growth often could not be measured directly because of nutrient depletion.

RESULTS AND DISCUSSION

One cannot specify any typical spectral distribution for sunlight in sea water as this depends on the depth, the attenuation properties of the water and the conditions of the sky. However, no form of daylight when viewed at a depth of more than a few centimetres resembles spectrally either unfiltered surface sunlight or the light obtained from the "cool-white" or "daylight" fluorescent lamps commonly used as sources of illumination for marine phytoplankton studies. In particular, light of wavelengths greater than 6500 A or less than 4000 A is almost completely missing from submarine light, as will be seen from the curves in Fig. 5 for sunlight in the clear sky shining through 5 m and 35 m of moderately clear coastal sea water. The spectral distribution of the filtered tungsten light used in this work is far from ideal. The peak is at too long a wavelength (although nearer the peak transmission of daylight in turbid coastal waters) and the base of the curve is too broad. However, this light is probably an adequate standard source for most purposes and, as mentioned earlier, it can be used for work at high intensities and is cheap and convenient to produce.

One might suppose the spectral quality of light to be significant in photosynthesis studies. The solid line in Fig. 5 shows the general shape to be expected for the action spectrum of a diatom (or dinoflagellate) plotted on an equal energy (not quantum) basis. The effect of wavelength on photosynthesis is obvious but the quantitative effect on cell division kinetics is less certain. Braarud (1937) noted an effect on *Skeletonema* growth and Lazaroff and Schiff (1962) have reported a spectral effect for the cell division of a blue-green algal. Baatz (1941) reported considerable differences in cell division rates and morphology when diatoms were exposed to lights of equal energy but different colours.

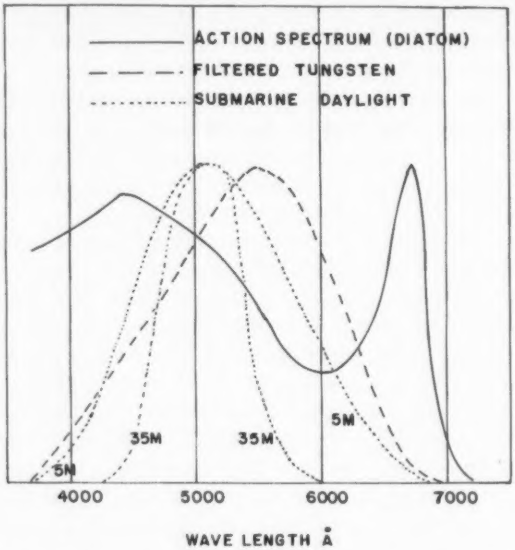
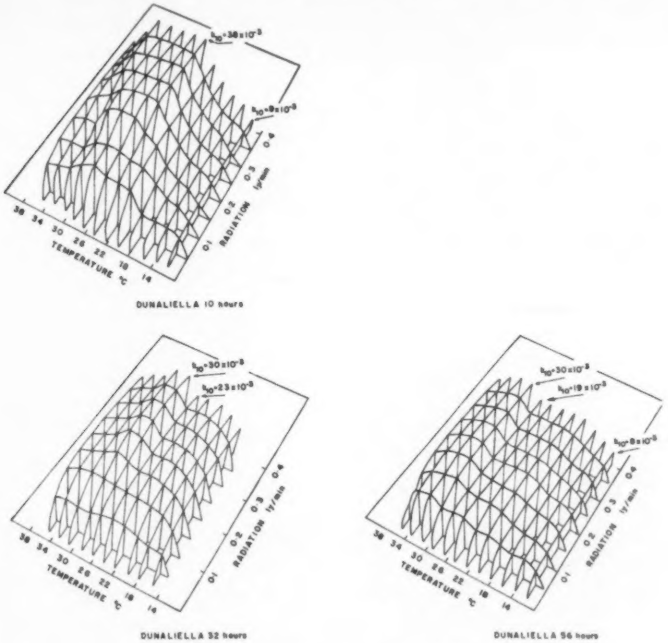


FIG. 5. Action spectrum (relative values for equal *energy*), submarine daylight and filtered tungsten light.

The results of experiments with 5 algae, one from the Chlorophyceae, one from the Dinophyceae, one from the Chrysophyceae and two from the Bacillariophyceae are shown by Fig. 6–10 inclusive. Quantitative data and some observations are collected in Tables I and II.

An unexpected result of the present work is the absence of supraoptimal light effects on cell divisions. Short-term experiments by Ryther (1956) had given the impression that the Chlorophyceae were particularly susceptible to suppression at high light intensity. This is clearly not the case with the cell division of *Dunaliella tertiolecta* or with any of the other 4 algae studied, which were selected from the more common algal divisions of the marine phytoplankton. The effects found by Ryther and others must arise either because of the duration of the experiment, the temperature, or because of the inhibiting effect of ultraviolet light in the unfiltered sunlight used by these workers to get high light intensities. Light of very short wavelengths may effect photosynthesis and give rise to short-term suppression such as is reported in some *in situ* marine productivity experiments. However, when these short wavelengths are eliminated cell division rates are not effected at intensities of 0.4 ly/min or higher. This is the intensity of photosynthetic radiation to be expected beneath only a few metres of sea water on a bright summer day (very approximately 75 kilo-lux as measured by most light-meters).

The minimum light intensity for cell division in each species is not known precisely as it was obtained by extrapolation, but the range in which the value lies is given in Table II together with the temperature range over which the value

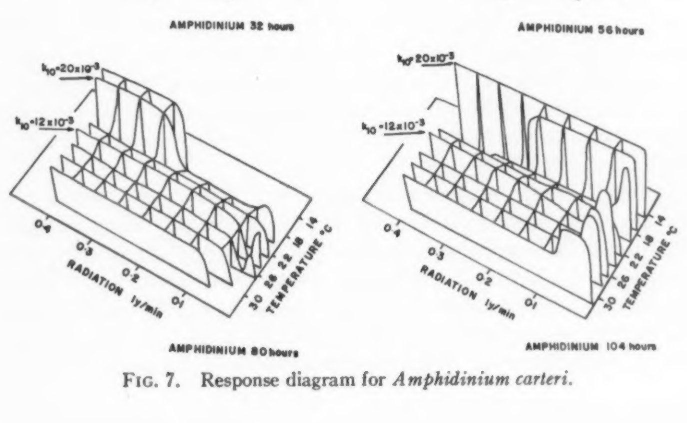


DUNALIELLA 10 hours

DUNALIELLA 32 hours

DUNALIELLA 56 hours

FIG. 6. Response diagram for *Dunaliella tertiolecta*.



AMPHIDINIUM 32 hours

AMPHIDINIUM 56 hours

AMPHIDINIUM 80 hours

AMPHIDINIUM 104 hours

FIG. 7. Response diagram for *Amphidinium carteri*.

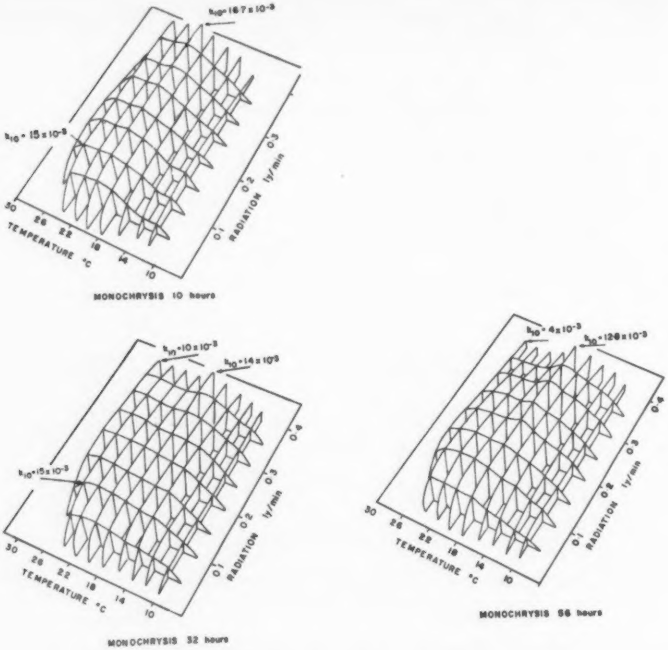
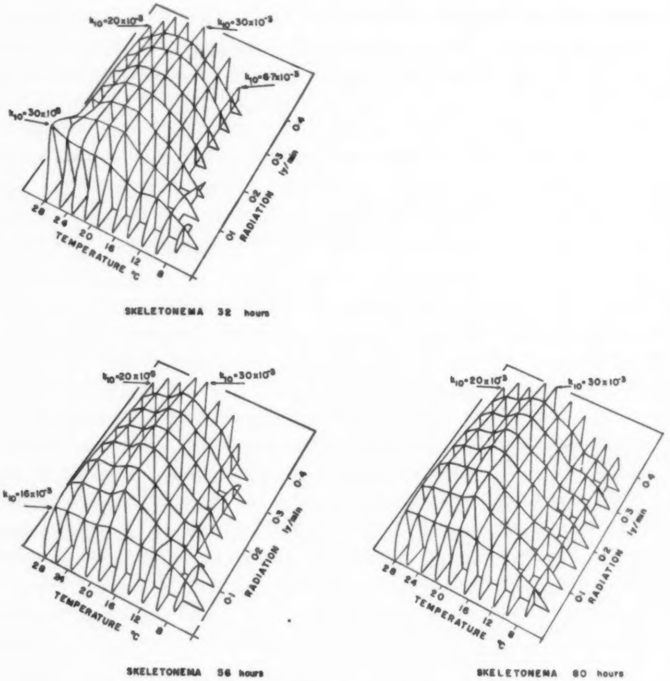


FIG. 8. Response diagram for *Monochrysis lutheri*.

was at a minimum. The 2 diatoms needed the lowest illumination. Significantly more light was required to cause division in the 3 flagellates, the natural habitat of which would have been near the sea surface or in pools and marshes.

An optimum light and temperature for the steady rapid growth of the species is suggested in Table I. Such conditions would prove ideal for culturing. The maximum value for the rate constant and the conditions under which this is found are given in the adjacent 2 columns. These rates have no absolute significance, as exact values depend on the light–dark cycle and on the chemical composition of the media, but they are thought to be near to the maximum. A better medium, more agitation of the cultures and a longer lighted period might have shortened division times substantially but a separate investigation is needed to study these effects. The present response diagrams illustrate the general behaviour of each species.

The extremes of temperature and light at which any cell division at all will take place are also shown in Table I. No upper limit of light intensity was found. At optimum temperatures growth was not impeded in any species at 0.4 ly/min or over 0.5 ly/min in the case of *Monochrysis lutheri* and *Thalassiosira nordenskiöldii*. It is remarkable what high intensities can be tolerated provided that temperatures are suitable. ***Thalassiosira nordenskiöldii*** was still dividing at 2°C, *Monochrysis lutheri* at 27°C, and ***Skeletonema costatum*** at 28°C but other experiments indicated that these temperatures were all within 2°C of the growth limit. The upper limit for *Dunaliella tertiolecta*, however, is unknown and probably exceeds 38°C (Ukeles, 1961).

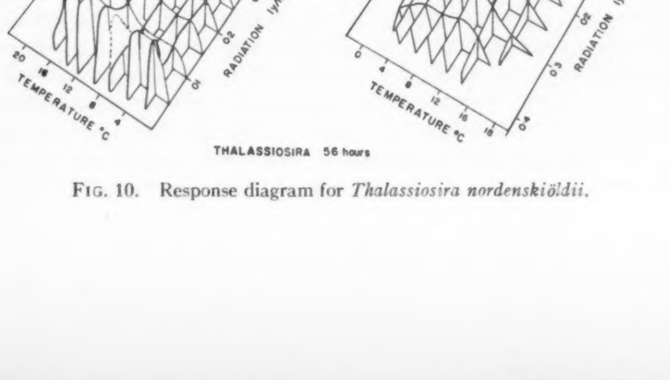


SKELETONEMA 32 hours

SKELETONEMA 56 hours

SKELETONEMA 80 hours

FIG. 9. Response diagram for *Skeletonema costatum*.



THALASSIOSIRA 56 hours

FIG. 10. Response diagram for *Thalassiosira nordenskiöldii*.

The temperature ranges for *Dunaliella* and *Monochrysis* are not unreasonable considering the natural habitat of these organisms and it may be significant that the dinoflagellate ***Amphidinium carteri*** has the narrowest range of all. The maximum growth temperature for the 2 diatoms is higher than they would normally encounter in nature. One would have expected much better growth at lower temperatures. This is particularly true in the case of *Skeletonema* which flourishes in late winter in Long Island Sound in temperatures of 3–4°C but which could not be induced to divide below 6°C in the present work. This was not due to the organism being killed by thermal shock. The same anomaly has been remarked upon by Ryther and Guillard (1962). Either some substance is missing from the culture media or perhaps a "low temperature" and "high temperature" strain of *Skeletonema* may be produced as a result of auxospore formation in a critical temperature–light regime (Guillard, personal communication).

The broad tolerance of *Skeletonema* for light and temperature variations is in keeping with its ubiquitous occurrence in coastal areas at all times of the year. The rather complex growth pattern of *Thalassiosira nordenskiöldii*, shown in Fig. 10, makes plain why this diatom has been seen in both early-spring and mid-summer plankton. The diatom is characteristic of cold-water, early-spring phytoplankton and is generally one of the first diatoms to appear in the "Spring Bloom". It is soon displaced in abundance by *Skeletonema* which Conover (1956) attributes to temperature effects. It will be seen from Fig. 10 that *T. nordenskiöldii* grows well in cold water but only at low light intensities (we suspect the absence of a peak at 7°C to be an experimental error). Its loss from the spring phytoplankton may thus be due to an increase in mean light intensity rather than to a temperature rise. The diatom also grows well at much higher light intensity but only when the water temperature has increased (Braarud, 1937) which could explain its appearance as a major constituent of summer phytoplankton in water of 13°–16°C (McAllister *et al.*, 1961).

The most remarkable feature of the response of these organisms to temperature is the relatively low Q_{10} for cell division, especially over certain ranges of illumination. This is illustrated in Table I where the temperature range over which growth constants are greater than two-thirds of the maximum are reported, that is the range in which a growth constant does not differ from a mean value by more than about 20%. This range is comparatively narrow (6°C) with *Thalassiosira nordenskiöldii* (compare the coccolithophore, *Coccolithus huxleyi* studied by Mjaaland, 1956) but is over 10°C for the other species, being as much as 13°C for *Skeletonema* (Curl and McLeod, 1961) and 17°C for *Dunaliella*. This behaviour is in marked contrast with the reported response of phytosynthetic rates to temperature and light (Curl and McLeod, 1961, for *Skeletonema*) but was observed by Spencer (1954), McCombie (1960), and Kain and Fogg (1960) for cell division. Such a wide temperature range suggests a self-adjusting enzyme system as postulated by Hastings and Sweeney (1957) to explain the temperature independence of diurnal luminescence. If cell division rates are less variable than is photosynthesis there should be size variations between cells in different parts of the cross gradient. No attempt was made to measure size changes but they were visually obvious in the case of *Skeletonema* (Table II) and indicated that at low temperatures photosynthesis increased more rapidly than did cell division as the light intensity increased. Auxospore formation was not noted during the formation of these large cells but could have been missed.

The "plateau" of near-constant rates dropped very rapidly at the high temperature limit, possibly due to a sudden increase of metabolite requirements (Hutner *et al.*, 1957). Heat damage to cells was largely irreversible. At lower temperatures the decrease of rate constant was less dramatic and cells were able to recover from moderate cold shock (Ukeles, 1961).

A further aspect of the response diagrams shown in Fig. 6–10 merits discussion. At either high or low temperatures, and sometimes both, there was evidence that an unfavourable illumination adversely effected cell division, the same intensity causing no trouble when temperatures were less extreme. A "stress pattern" emerged which is summarized in Table II. This effect has been noted with *Chlorella* division by Sorokin and Krauss (1962) and has been discussed at length to explain some aspects of the succession of freshwater phytoplankton species by Findenegg (1943), Rodhe (1948) and Vollenweider (1950). The phenomenon is well illustrated by the behaviour of *Thalassiosira nordenskiöldii* where good growth was possible at low temperatures only with low light intensities or at high light only within a comparatively narrow temperature range. Species succession is favoured not only when the water temperature rises above the optimum range for one species into the optimum range for another but these optima themselves depend on the mean light level.

A very striking example of stress was discovered when cultures of *Monochrysis lutheri* and *Thalassiosira nordenskiöldii* were accidentally poisoned chemically (the use of rubber corks in the first case and for unknown causes in the second case). In neither case was the poison in the media sufficient to kill the organisms outright but growth was restricted to tubes in one part of the block which coincided almost exactly with the light–temperature optima (Tables I and II). The "chemical stress" added to those of temperature illumination prevented any growth elsewhere. The ecological implications of these observations are clear if we assume that the presence of undesirable substances or a shortage of certain growth factors in the natural environment produce, in effect, a "chemical stress" on the organism which narrows its light–temperature tolerance.

A very marked outburst of growth occurred in the cultures of *Amphidinium carteri* under conditions of light and temperature just outside that which seemed to be the normal growth areas (Fig. 7). Rapid cell division occurred over a period of about 24 hr and then stopped again. These temporary "outbursts" occurred after some 60 hr and started first in areas of high light and low temperature followed by the cells subjected to low light at both low and high temperatures. The cell increases were too great for the behaviour to be attributed to experimental error but no explanation can be given unless the cells in these regions were in an unstable "resting stage".

The present technique of measurement, using 64 different combinations of light and temperature, has been applied to 5 phytoplankton cultures to illustrate the use of the apparatus and detailed behaviour of species grown over a period of 4–5 days. The apparatus can be used by one man but the method is tedious and time-consuming. It would probably be better to fill the block with tubes all at the temperature of the stock culture solution and then slowly impress the desired temperature gradient over a period of 10–20 hr rather than put the cultures directly into tubes at widely differing temperatures, as done in the present experiments. A more rapid but doubtless adequate picture of cell division kinetics could then be obtained from only 2 counts from each tube, one after about 10–20 hr and the second one some 36–40 hr later. The results would give only an integrated view of the division rates over the period concerned but would be a useful indication of the response of the organisms.

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Marine Phytoplankton Photosynthesis as a Function of Light Intensity: A Comparison of Methods[1]

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ABSTRACT

An apparatus is described in which the constant for exponential algal growth has been determined as a function of light at controlled temperature. Studies were made of bacteria-free cultures of *Skeletonema costatum* and *Dunaliella tertiolecta* grown at the optimum temperature for cell division in media with and without adequate nitrate and phosphate. Less complete studies were made of the behaviour of *Monochrysis lutheri* and *Amphidinium carteri*. In all experiments the growth constants were determined at known light intensities, expressed in the energy units, langlies/minute, using light of a known spectral distribution similar to sunlight shining through a few metres of coastal sea water.

Growth constants were determined for the following processes: increase of cellular carbon, chlorophylls *a*, *b* or *c*, carotenoids, cell numbers and photosynthesis measured both by oxygen evolution (net and gross) and by the uptake of C-14 labelled carbonate.

The rate of maximum photosynthesis occurred when the illumination reached about 0.1 ly/min and little or no light inhibition occurred when the intensity was increased to as high as 0.4 ly/min. Respiration was about 10% of maximum gross photosynthesis. This fraction increased when cells were nitrogen deficient but decreased in phosphate deficient cultures because a phosphate shortage inhibited respiration more severely than photosynthesis.

Gross photosynthesis at low light intensities was proportional to the *total number of molecules* of all plant pigments added together, irrespective of species or of culturing conditions. By contrast, the rate of maximum gross photosynthesis was poorly related to pigment composition, the best correspondence being with the amount of chlorophyll *a* in the cultures.

The uptake rate of C-14 varied with time. There was an apparent "leakage" of labelled organic matter which eventually reached a near-equilibrium with $^{14}CO_2$ uptake, after which the C-14 method measured the production of particulate carbon but not necessarily net or gross photosynthesis. The rate of particulate carbon production was the same as that of cell division but about 40% less than net photosynthesis in cultures of *Skeletonema*. Agreement between C-14 rates and net photosynthesis was better in cool-white fluorescent light than in the imitation submarine sunlight used in this work. At high light intensities there was apparently no excretion from *Dunaliella* "shade" cells but when these became changed to "sun" cells they behaved more like *Skeletonema*.

Various observations are recorded of the effect of growth conditions on cell composition, especially with regard to pigments.

The relatively low C-14 rates found with *Skeletonema costatum* may be explicable *in part* by an abnormally low counting efficiency for this species even when present as a "weightless" source but the problem requires further study.

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INTRODUCTION

DESPITE the large number of studies of the effects of light on the photosynthesis of unicellular algae comparatively few have been made with marine phytoplankters (Strickland, 1960, for a review) especially unialgal bacteria-free cultures where the respiration of the plants can be assessed. In many studies (Jenkins, 1937; Ryther, 1956; Ryther and Yentsch, 1957; Talling, 1960; Curl and McLeod, 1961) photosynthesis has been studied by only one method, either the "light and dark bottle" oxygen technique or the more popular "C-14" method which measures the uptake of radioactive carbonate. Where both methods have been used simultaneously on cultures (Ryther and Vaccaro, 1954; Nielsen and Al Kohly, 1956; Ketchum *et al.*, 1958; Smith *et al.*, 1960; McAllister, 1961; Thomas, 1963) agreement between them has sometimes been good and sometimes bad. With natural populations the agreement is often poor (Ryther and Yentsch, 1958; McAllister *et al.*, 1961; Antia *et al.*, 1963). The C-14 method can give results very much lower than those calculated from oxygen evolution and there is evidence from the work of Antia *et al.* (1963) that C-14 uptake measures the increase of *particulate* carbon in a mixed diatom bloom, and this may be less than 70% of the net or gross photosynthesis.

It is now essential to have unequivocable evidence as to what is being measured by the various techniques which can be employed in marine productivity work. This is not forthcoming from existing studies with pure cultures. Most of these have been made using freshwater Chlorophyceae, notably the various *Chlorella* species, and it seems unwise to assume that the same physiology will be found in organisms as far removed morphologically from these algae as are, say, the marine diatoms. Furthermore, the spectral quality of the light used for quantitative kinetics is important and it is difficult to interpret the results which have been obtained using light sources greatly dissimilar to submarine sunlight, especially when intensities have been reported in relative units such as foot-candles.

In the present investigation new equipment has been designed to measure photosynthesis at a controlled temperature using a wide range of light intensities of known spectral composition and energy. Growth has been measured in six different ways simultaneously on bacteria-free cultures of a few marine phytoplankters, recording oxygen evolution, C-14 uptake, and the increase of cell numbers, chlorophylls, carotenoids, and particulate carbon. Measurements have been made on cultures containing excess nutrients and on cultures depleted in either nitrate or phosphate. Conditions are "artificial", because of the use of axenic cultures of high density in enriched sea water, but nevertheless it is reasonable to suppose that the quantitative data found in these experiments has some relevance to natural phytoplankton growth, which cannot be measured or interpreted with such certainty.

EXPERIMENTAL METHODS

CULTURES AND MEDIA

The same bacteria-free cultures of *Dunaliella tertiolecta*, *Monochrysis lutheri*, *Amphidinium carteri*, and *Skeletonema costatum* were used as in the Jitts *et al.*

(1964) paper. The basic medium for all experiments was taken from a large stock of coastal sea water from a depth of 20 m in Departure Bay, Nanaimo, British Columbia. It was filtered through a membrane filter before storage to remove all algae and re-filtered through an HA Millipore again immediately before use. The salinity of the water was 29‰, it had a specific alkalinity of 0.125. Initially about 22 μg-at NO_3–N/litre, 2 μg-at PO_4–P/litre and 50 μg-at Si/litre were present.

Cultures were made from this water in 10-litre quantities, all solutions being enriched by 1 ml/litre of a solution of EDTA-chelated trace metals and 1 ml/litre of a solution of growth factors. The composition of both solutions is given in Jitts *et al.* (1964). Suitable additions of nitrate were made from potassium nitrate solution, phosphate from a disodium hydrogen phosphate solution (pH about 8) and silicate from a neutralized sodium meta-silicate solution. The medium was placed in a 12-litre glass bottle fitted with a foil-covered cork through which passed a glass tube and a cotton-wool plugged exit tube for air. The glass tube reached to the bottom of the bottle and was connected outside to a length of silicone rubber tubing and a small cotton wool air filter. The whole assembly was sterilized for 20 min at 15 psi of steam. No precipitate formed if phosphate was sterilized separately and added after solutions had cooled. The culture medium was aerated vigorously for several hours to bring the pH to 8.0–8.1 before it was inoculated with a small volume of algal culture, previously grown to be in an exponential phase. Tests for bacteria were made at intervals during culturing and were always negative.

METHODS OF ANALYSIS

NUTRIENTS. Nitrate and silicate were analysed on filtered solution as described by Strickland and Parsons (1960). The presence of EDTA gave a slight but unpredictable interference with the nitrate method which was therefore standardized internally. Phosphate was estimated on filtered solutions using the antimony-catalysed method described by Murphy and Riley (1962).

TOTAL CARBON DIOXIDE. This was estimated from the salinity, total alkalinity and pH using tables given by Parsons and Strickland (1960).

CELL COUNTS. Counts were made in a hemocytometer, counting replicates until between 500 and 1000 cells had been recorded, thus giving counts with a coefficient of variation of about 3–4%.

PIGMENTS. The Richards with Thompson method (1952) was used with a modified equation suggested by Parsons and Strickland (1963). Whatman G.F/C glass papers were employed to reduce turbidity blanks. A comparison of results obtained with Millipore HA, Millipore AA and the glass filter showed no significant difference if the suction vacuum was about $\frac{1}{3}$ atmosphere. In the Parsons-Strickland equations the quantities of chlorophylls a, b and c are expressed as milligrams. Carotenoids are still expressed in an arbitrary unit but this is thought to be sufficiently close to the milligram for the present purpose when account is taken of the different specific extinction coefficients of lutein, fucoxanthin and peridinin. For calculating the approximate number of moles of pigment in a culture the molecular weights of chlorophyll *a*, chlorophyll *b*, chlorophyll *c*, lutein, fucoxanthin and peridinin were assumed to be 900, 900, 1100, 600, 650 and 650, respectively.

CELL CARBON. This was determined using the dichromate-oxidation method described by Strickland and Parsons (1960). Results for 3 of the species are lower than the true carbon figures by about 10–15% (Parsons *et al.*, 1961). No correction was made, however, except for the analysis of the fourth species, *Monochrysis lutheri*, where the oxidizable carbon results were adjusted to make them comparable with those of the other three. Determinations of carbon increase were not made in cultures deficient in nutrients as growth was too slow.

PHOTOSYNTHESIS BY OXYGEN EVOLUTION. Oxygen was determined by the Winkler method and results calculated as described by Strickland and Parsons (1960). Precision was uniformly excellent and results are considered to be generally better than ±5 mgC/m³/hr. Respiratory quotients have been assumed to be unity. The correct value for the photosynthetic quotient $\frac{+\Delta O_2}{-\Delta CO_2}$ is particularly relevant to this work. From direct analysis of the plants concerned (Parsons *et al.*, 1961) one would expect a maximum value of about 1.3–1.4 for all species when growing with nitrate as nitrogen source. A value of 1.25 was finally chosen to ensure that C-14 results did not exceed the gross photosynthesis rates measured by oxygen changes in any experiment. In experiments with nitrate absent the PQ was assumed to be unity although it may have been slightly higher. PQ values undoubtedly vary from species to species and may depend on light intensity but it is most unlikely that the correct values differed from the value chosen by more than 10%.

UPTAKE OF RADIOCARBON. The technique used and method of calculation were as described by Strickland and Parsons (1960) with slight modifications. Either 1 or 5 microcuries of activity were added to 300-ml bottles so that the final counting rates on filters were in the range 200–10,000 counts/min. The volume of solution filtered was chosen so that the total "thickness" of algae did not exceed 0.1 mg/cm² of filter surface and was normally 0.05 mg/cm² or less. No self-absorption corrections were made. Both formalin preservation and hydrochloric acid treatment were omitted to lessen the chances of radioactive loss from damaged cells. Suction pressures were automatically controlled so as not to exceed ⅓ atmosphere. Filters were rinsed with small volumes of sea water. "Dark blanks" were determined but were negligible except with a few of the lowest counts. The total radioactivity added was determined by scintillation counting and the efficiency of the counter (Baird Atomic, with thin Mylar end-window) by the Jitts and Scott method (1961).

PRECULTURING

The 12-litre glass bottle of culture was placed in a thermostatically controlled water bath at the desired temperature and illuminated with a bank of cool-white fluorescent lights. The mean intensity of illumination was difficult to estimate because of the size and shape of the assembly but did not exceed 0.05 ly/min of photosynthetically active light as measured by a thermopile radiometer.

The culture was aerated vigorously with filtered and humidified air, and grown for at least 24 and generally more than 48 hr until the desired cell density for the main experiment was achieved. Aliquots were withdrawn, aseptically, at intervals and analysed for pigments and cell counts. Growth was logarithmic for at least a day before the main experiments were commenced.

The media for most experiments contained 500 μg-at NO_3N/litre, 50 μg-at P/litre and, for *Skeletonema* only, 500 μg-at Si/litre. Growth was at substantially the same rate in such a medium as when nitrate, phosphate and silicate were at natural sea water levels (Barker, 1935) but was somewhat reduced if one nutrient was present in high concentration and the other in low concentration. For experiments designed to produce nitrogen- or phosphorus-deficient cells the appropriate nutrient was omitted and the plants allowed to strip this element from the sea water, concentrations being determined chemically at regular intervals.

Preculturing experiments are illustrated by Fig. 1 and 2 which show *Skeletonema costatum* growing in sea water with no extra added phosphorus and no added nitrogen, respectively. Growth was at first exponential and then rates, as measured by both pigment and cells, decreased when there was a

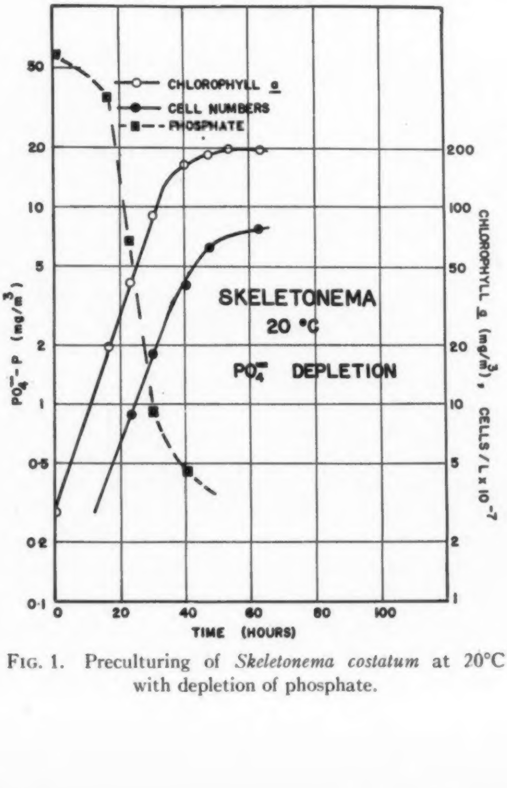

FIG. 1. Preculturing of *Skeletonema costatum* at 20°C with depletion of phosphate.

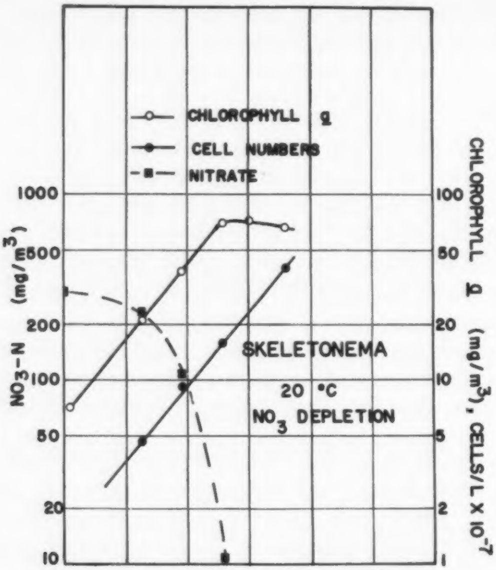

FIG. 2. Preculturing of *Skeletonema costatum* at 20°C with depletion of nitrate.

phosphorus deficiency. By contrast, cell division continued unchanged for nearly 2 divisions after nitrate had been stripped from the medium. The cell concentrations reached in these experiments and in the corresponding experiments with *Dunaliella* were undesirably high and a suitable fraction of the medium was therefore removed from the preculturing vessel and filtered aseptically through HA Millipore membrane filters. The filtrate was returned to the remaining culture and the whole re-aerated for about an hour before use. From the measured loss of nutrient and increase in cell numbers during preculturing the mean phosphorus or nitrogen content of the cells could be estimated at various times. Direct analysis for particulate nitrogen confirmed these results.

THE APPARATUS AND ITS USE

The temperatures used for each species in the preculturing and main experiments were the optimum for cell division as determined in Jitts *et al.* (1964).

Cultures were filled into sterilized 300-ml BOD bottles for the main experiments. "Dark-bottle" controls were used for both the oxygen and C-14 methods and the initial oxygen content of the water was measured in bottles filled at the beginning and at the end of the sampling for oxygen. These two values were always in excellent agreement.

Samples for pigment, cell number and plant carbon analysis, each in duplicate, were taken at the same time as the BOD bottles were filled. The pigments, cell numbers and plant carbon content of samples exposed for either 6 or 10 hr to light of known intensity were determined from water in the BOD bottles but volumes were generally only sufficient for single determinations.

A specially designed incubator held the BOD bottles lying on their sides in black painted compartments in aluminum trays. The black painted trays rested on a layer of unpainted ones in which "dark bottles" could be placed. Perforations in the base of the trays allowed water to circulate up over the bottles and into overflow conduits from which it was pumped into a thermostatically controlled heater-refrigerator unit and recirculated. A temperature variation of less than ±1°C was achieved over a range of 25°C either side of ambient temperature.

Near-parallel light was directed vertically downwards onto the bottles from 5 Appleton Unilet units fitted with 500-watt General Electric T 3Q tungsten-iodine vapour lamps. The light was filtered through a Perspex filter of rapidly flowing cold water to remove heat and then through 2.0 cm of a chemical filter. The filter and spectral characteristics of the resulting light have been described in Jitts *et al.* (1964).

Two incubator units were connected in parallel to the thermostated water supply. A "high light" unit, which contained 10 illuminated bottle compartments with the lamps as near to the filter assembly as possible and a "low light" unit in which 5 lamps some distance above the filters illuminated 20 compartments. A view of the top of the "high light" unit is shown in Fig. 3. Light

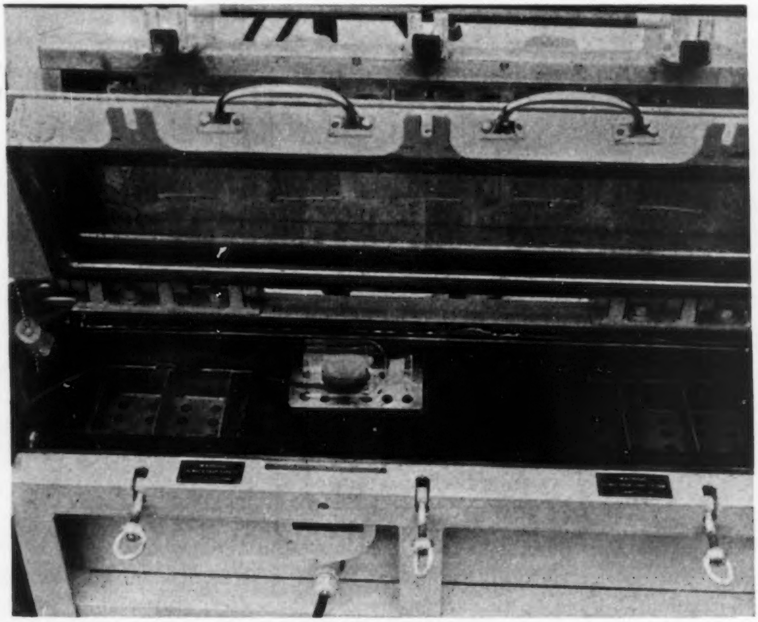

FIG. 3. High-light unit of incubator.

from lamps in the hinged lid (shown swung back to the vertical in the picture) passed through the cooling water and filter units (both in the hinged box with handles) on to bottles in the compartmented trays. One blackened tray is seen resting on the lower unpainted trays.

Light was measured by a gauze-masked Weston cell placed centrally in a BOD bottle as seen in Fig. 3. The output from this assembly filled with water and placed in each compartment gave the illumination for each section and the cell was left in one compartment during every experiment to monitor the light. The lamp voltage was controlled manually when necessary to ensure a constant illumination. The Weston cell was calibrated directly against a thermopile radiometer, also fitted centrally in a water filled BOD bottle, by placing both bottles alternately in the same compartment. In this manner the illumination in each bottle in the incubator was recorded in cals/cm^2/min (ly/min) of energy. The light distribution changed very little with time but was redetermined at suitable intervals. Intensities of 0.3–0.4 ly/min were obtained in the "high light" unit and between 0.1–0.15 ly/min in the "low light" unit. The illumination was reduced when necessary by covering individual bottles with a mesh filter described by McAllister and Strickland (1961) using fine nylon or monel mesh. The attenuation of light by each filter was measured with the Weston cell in the BOD bottle.

Bottles containing samples for photosynthesis measurement by the oxygen and C-14 techniques were placed in the incubator at suitable light intensities for exactly 3 hr. Samples used for measuring growth were illuminated for either 6 or 10 hr. During 3-hr periods there was no indication of cells settling appreciably in the bottle. Culture densities were too low for cells to attenuate the light appreciably.

Calculation and Presentation of Results

The rate of carbon uptake over a short period (t hours) represented as $\frac{\Delta C}{t}$ closely approximates the differential $\frac{dC}{dt}$ at time $\frac{t}{2}$, provided that the fractional increase in the population over the time interval t is not more than about 25%. These conditions apply to the present work and rates are therefore expressed as the differential rate 1.50 hr after the commencement of incubation. The value of $\frac{dC}{dt}$ depends on the culture density and cannot be used for comparisons. Results are therefore all expressed by rate constants, k, in the expression:

$$\frac{dC}{dt} = kC$$

where k has the unit (hours)$^{-1}$ and C is expressed in mgC/m^3 after 1.50 hr incubation. The concentration of carbon at zero time was determined chemically. The incremental increase in carbon by 1.50 hr, often appreciable, was then estimated from the C-14 uptake rates at each light intensity, as this was found to be *approximately* equal to the increase of particulate carbon (see later). Errors arising from this assumption were second order.

The measurement of k values at optimum temperatures is the best method for comparing the inherent growth rates of various algal species as a function of the culture medium and light intensity. The constant k is independent of concentration units but depends on the quantity being measured, being different for various cell growth processes if they occur at different rates in the same organism. A comparison of k values is thus a convenient method for deciding if the composition of a cell is changing with time. In Fig. 6–10, lines have been used to record k values for photosynthesis at 1.5 hr as measured by oxygen changes and C-14 uptake. Various symbols are used for the k values for cell division, pigments and cell carbon increases, measured over the designated time intervals. These were calculated assuming that the expression,

$$(\text{Property})t_2 = (\text{Property})t_1 \times \exp\,(kt_2 - kt_1)$$

could be applied over these time intervals.

The rate of C-14 uptake with time was not constant. In each experiment aliquots were taken at intervals from bottles exposed to low and high light intensities and an *average* rate of uptake was then calculated over periods of 2, 3, 6 hr, etc. Results from two experiments with *Dunaliella* and *Skeletonema* are shown in Fig. 4. The mean rate of uptake decreased for about the first

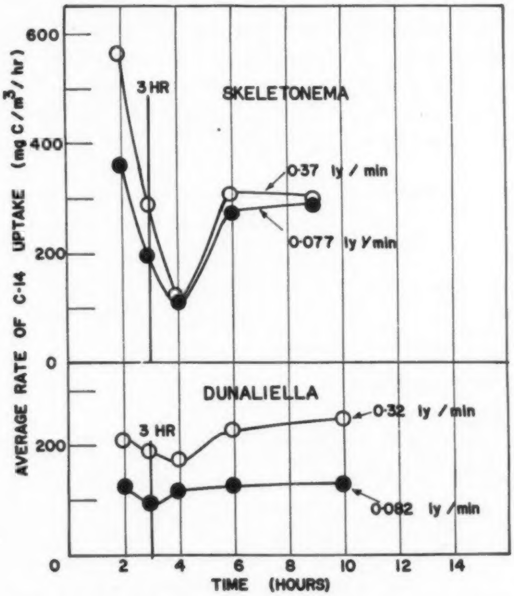

FIG. 4. Average rates of C-14 uptake over various time intervals in experiments with *Skeletonema costatum* and *Dunaliella tertiolecta* with high concentrations of nitrate and phosphate.

3–5 hr and then increased to a fairly steady value. The variations were worse at low light intensities and very bad in the case of *Skeletonema* grown in a medium with high concentration of nutrients. The effect was still noted but was very much less when the concentrations of nitrate and phosphate were at natural sea water level, the very high initial rate being much reduced. This behaviour was not studied further as it would have required a separate investigation but it appears as if some of the labelled carbon first taken up with the cells is rapidly excreted until an equilibrium or near equilibrium situation prevailed. The rates at 3 hr, used in the present work, were sometimes lower than the "steady" values. The discrepancy was worse in *Skeletonema* where *mean* rates at 3 hr were some 20–30% too low. All other 3-hr rates were more acceptable but it should be remembered that *k* values reported here for C-14 rates at 1.5 hr are the values expected had C-14 uptake rates been reasonably constant from the start and near to the "stable" values. The actual rates at 1.5 hr would, in most cases, have been higher.

In contrast with the C-14 data, photosynthetic rates measured by oxygen evolution remained constant for many hours except for the anticipated increase with time brought about by an increase in cell concentration due to growth in the culture.

The raw data for gross and net photosynthesis and C-14 uptake as a function of light intensity were plotted to give curves such as that shown in Fig. 5 for *Skeletonema costatum*. The best curve through the points was hand-drawn and rates read from this curve. The gross photosynthesis curve was made to pass through the origin. The net photosynthesis curve was drawn parallel to the curve for gross photosynthesis, it being assumed that the respiration measured in a dark bottle applied at all light intensities. This assumption is doubtful

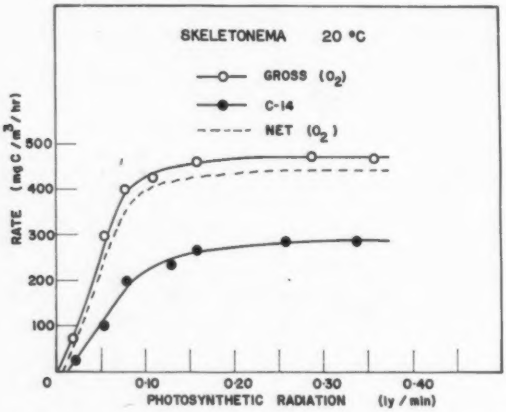

FIG. 5. Experimental data for an experiment with *Skeletonema costatum* in a medium with high concentrations of nitrate and phosphate.

but in most experiments likely variations would probably not affect the general picture. Raw data plots were satisfactory in all experiments but, because of space consideration, only the example in Fig. 5 is presented, the other data being summarized by graphs showing only smoothed *k* value.

RESULTS AND DISCUSSION

k values as a function of light intensity are collected in Fig. 6–10 which summarize results from the main experiments except for certain checks and an experiment with *Skeletonema costatum* in sea water with no extra added nutrients, which gave results almost identical to those in Fig. 10 except that C-14 uptake rates were slightly less. The experiment with *Dunaliella* at low phosphate concentration (Fig. 6B) was started too soon and although cells had minimal phosphorus they behaved normally in many respects and were not really deficient in phosphorus as in the corresponding *Skeletonema* experiment. For this reason

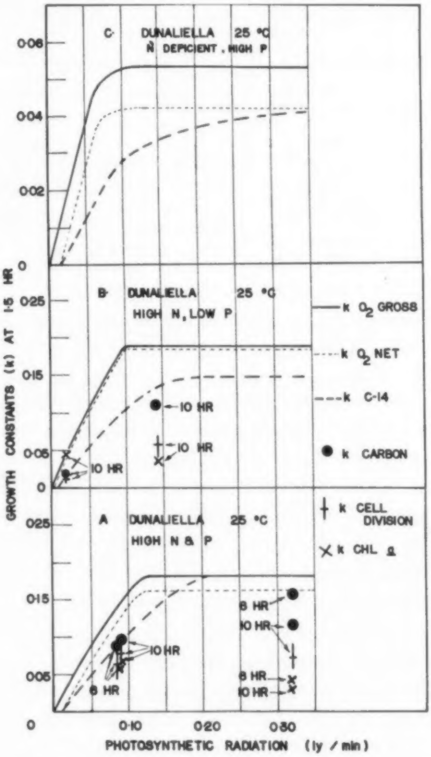

FIG. 6. Rate constants (base e) for *Dunaliella tertiolecta*, experiments at 1.5 hr or mean values over period indicated.

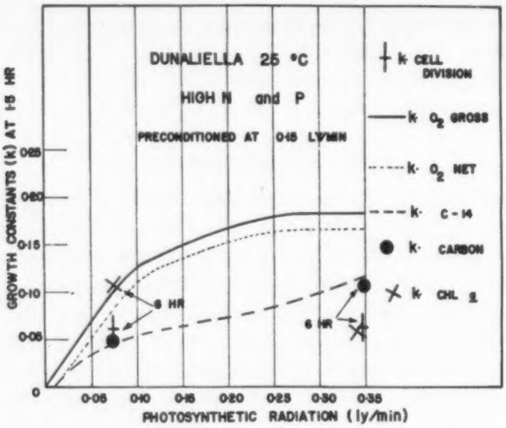

FIG. 7. Rate constants (base e) for *Dunaliella tertiolecta*, (preconditioned at 0.15 ly/min) at 1.5 hr or mean values over period indicated.

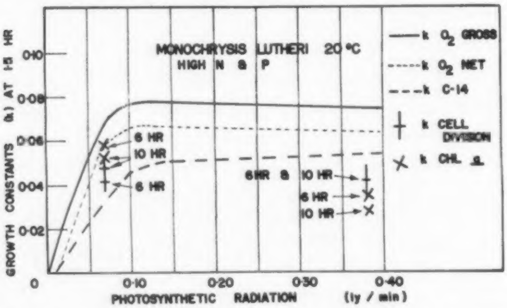

FIG. 8. Rate constants (base e) for *Monochrysis lutheri* at 1.5 hr or mean values over period indicated.

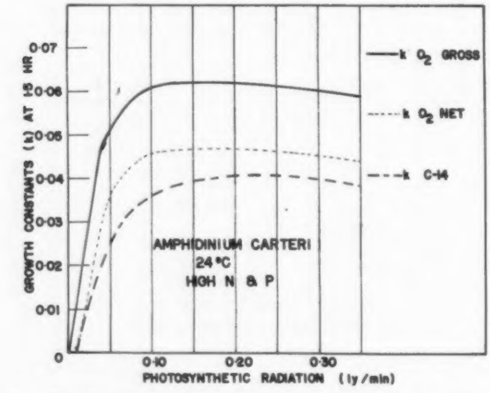

FIG. 9. Rate constants (base e) for *Amphidinium carteri* at 1.5 hr.

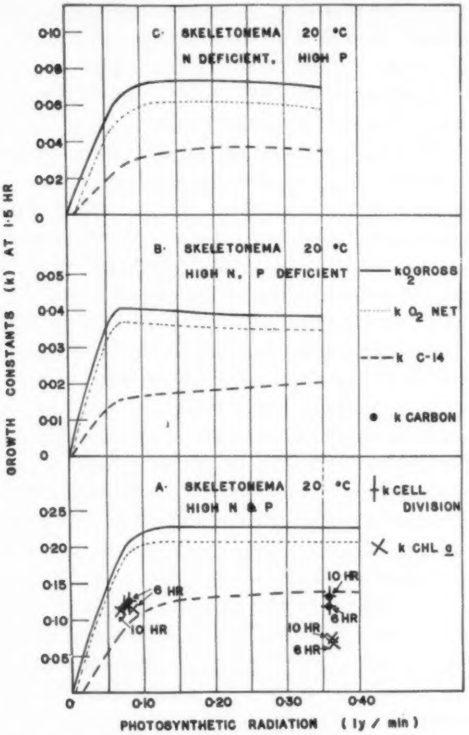

FIG. 10. Rate constants (base e) for *Skeletonema costatum* at 1.5 hr or mean values over period indicated.

the term "low" rather than "depleted" has been used. In the *Skeletonema* experiment (Fig. 10B) phosphorus in the cells was reduced to only about a tenth of its normal value. In both experiments with nitrogen deficiency (Fig. 6C, 10C) cells were seriously depleted and contained only a quarter of the normal amount of this element. During preculturing the growth rate of *Skeletonema* became affected when the phosphorus in the medium decreased below about 0.03 μg-at P/litre and the nitrate below about 0.5 μg-at N/litre.

The *k* values for respiration are collected in Table II and were similar for all algae (0.01–0.02) and only about 10% of the maximum rate of gross, photosynthesis. Nitrogen deficiency lowered the rate of respiration but had a greater affect on photosynthesis so that respiration was increased to nearly 20% of gross photosynthesis. It is interesting to note the very marked effect that the phosphorus deficiency has on reducing the rate of respiratory processes as compared with photosynthesis. In the *Dunaliella* culture with "low" phosphorus, photosynthesis was unaffected but respiration was reduced to a fifth of its normal value making maximum net and gross photosynthesis virtually identical.

The compensation intensities for photosynthesis (I_{comp}) were estimated from the net photosynthesis curves and were approximately the same as for cell division (Jitts *et al.*, 1964). Values for I_{comp} and for respiration can be obtained from C-14 curves according to Nielsen and Hansen (1959a). I_{comp} results were generally similar when obtained by their method.

The intensity of illumination at which *k* values for gross photosynthesis first reached a maximum (I_{max}) cannot be determined precisely though it was very near to 0.1 ly/min for all cultures except the one conditioned at high light (see later) or deficient in phosphorus (Table I). Most surprisingly there was no significant decrease of photosynthesis at high light intensities. Even at 0.4 ly/min the decrease did not amount to more than 5% and in most experiments no decrease at all could be detected and a "plateau" extended from about 0.1–0.4 ly/min. This confirms the results found previously with cell division rates at optimum temperatures but is quite contrary to what would be expected from reports in the literature (Jenkins, 1937; Ryther, 1956; Curl and McLeod, 1961) and for some observations of photosynthesis profiles measured *in situ* in the euphotic zone. The explanation may lie in the absence of potentially inhibiting ultraviolet radiation from the light source (Redford and Myers, 1951) but could be because all cultures were conditioned at their optimum temperatures. Supra-optimal light inhibition in nature may well depend on how near the species present are to their optimum growth conditions.

k values vary from species to species and are reduced drastically when cells become deficient in nitrogen or phosphorus (Fig. 6 and 10).

It has been assumed by many that the rate of photosynthesis, P, in particular the rate at I_{max}, (P_{max}), can be related to the chlorophyll *a* content of cultures of natural populations. Values quoted for B in the equation,

$$\text{mgC/m}^3\text{/hr} = \text{B.mg chlorophyl } a\text{/m}^3$$

(where B = *k* divided by the cellular ratio of chlorophyll *a* to carbon) vary from about 2 to 8 (Strickland, 1960). With the precise data obtained in the present work one might expect to find a narrower range. Values for B calculated from gross photosynthetic rates at 0.30 ly/min (P_{max}) vary just about twofold (Table II) for 3 very different species with and without an adequate supply of nutrients. The range was considerably greater when C-14 rates were used. Such a variation, although small in terms of the precision used for field studies, is far from negligible. It does appear, however, that the enzyme systems that become saturated and limit photosynthesis at high light intensities are present *roughly* in proportion to the amount of chlorophyll *a* in the system. The relationship was not as good with any other pigment or combination of pigments. It was seen from Table II that the amount of cellular nitrogen is as good an index of P at 0.3 ly/min as is chlorophyll *a* in the 4 samples in which nitrogen data are available and it is possible that in many instances chlorophyll *a* is acting as a convenient rough index of cellular nitrogen. In view of the effect of preconditioning light intensities on the chlorophyll content of a cell and the absence of preconditioning effects on k_{max} one cannot expect B to be constant between "sun" and "shade" cells (see later).

TABLE I. Composition of algae with other data at commencement of kinetic experiments.

Algae and media		I (comp) ly/min	I (max) ly/min	$\frac{\text{Secondary Chl}}{\text{Chl } a}$	$\frac{\text{Carotenoids}}{\text{Chl } a}$	$\frac{\text{Carbon}}{\text{Chl } a}$	$\frac{\text{Chl } a}{\text{Cell}} \times 10^{12}$	$\frac{\text{Carbon}}{\text{Cell}} \times 10^{12}$	$\frac{\text{Nitrogen}}{\text{Cell}} \times 10^{12}$	$\frac{\text{Phosphorus}}{\text{Cell}} \times 10^{12}$
Dunaliella	High N									
	High P	0.013	0.125	0.18	0.328	22.3	1.9	42	5.3	1.1
(Low light preconditioning)										
Dunaliella	High N									
	High P	0.013	*ca.* 0.3	0.15	0.410	38	0.69	26.5	-	-
(High light preconditioning)										
Skeletonema	High N	0.006	0.125	0.37	0.91	25	0.59	15	2.7	0.60
	High P									
Skeletonema										
Natural sea water level of nutrients		0.006	0.125	0.37	0.92	28	0.60	17	*ca.* 3	*ca.* 0.8
Monochrysis	High N									
	High P	0.01	0.10	0.25	1.37	71	0.18	11.5	-	-
Amphidinium	High N									
	High P	0.01	0.125	0.80	1.65	54	2.0	108	-	-
Dunaliella	Low P	0.002	0.10	0.20	0.313	15.5	1.9	29	-	0.31
Dunaliella	N. deficient	0.015	0.10	0.19	0.46	57	0.74	42	1.33	-
Skeletonema	P. deficient	0.006	0.07	0.34	1.17	93	0.27	24.5	-	0.054
Skeletonema	N. deficient	0.012	0.10	0.33	1.18	68	0.165	11	0.70	-

TABLE II. Certain kinetic data in summary form. (P = gross rate of photosynthesis (in mgC/m³/hr) measured by oxygen changes.)

Algae and media		k(Resp)	Response % / Max. gross O_2	P / Cellular N (0.3 ly/min)	P / Chl *a* (0.3 ly/min)	P / Total moles pigment (0.3 ly/min)	P / Chl. *a* (0.03 ly/min)	P / Total moles pigment (0.03 ly/min)
Dunaliella	High N High P	0.019	8	1.4	4.85	2580	1.10	585
(Low light preconditioned)								
Dunaliella	High N High P	0.017	9	-	7.1	3600	1.29	660
(High light preconditioned)								
Skelotonema	High N High P	0.020	9	1.25	6.20	2060	1.78	620
Monochrysis	High N High P	0.011	13	-	4.70	1370	1.92	560
Dunaliella	Low P	0.0035	1.5	-	3.30	1760	0.93	490
Dunaliella	N deficient	0.011	20	1.7	3.85	1840	1.15	560
Skeletonema	P deficient	0.0040	10	-	3.50	1080	1.93	600
Skeletonema	N deficient	0.012	16	1.15	5.0	1500	2.18	650
Ratio of extreme values		-	-	1.48	2.15	3.33	2.35	1.35

At low light intensities (e.g. 0.03 ly/min) C-14 rates were again not related very well to quantities of pigment but the rate of gross photosynthesis was *proportional to the total number of moles of all plant pigments*. This relationship was very much better than the relationship to chlorophyll *a* or any other pigment or combination of pigments. Values vary only by a factor of 1.35 (Table II) which, considering the precision of data, the free-hand drawing of curves, the great differences of pigment composition in the cultures and the fact that the method used for pigment analysis did not give absolute weights of carotenoids, must be considered as evidence for a close quantitative relationship. Better results were obtained by using the number of pigments molecules rather than the total pigment weight but the improvement was only marginal and total weight would be adequate for all practical purposes.

It appears as if, at low light intensities, kinetic control depends only on the energy of illumination and the total number of molecules of pigment and is largely independent of the organism and its physiological condition. It might therefore be wise to estimate all pigments and not only chlorophyll *a* whenever possible in field studies (Currie, 1958; McAllister *et al.*, 1960).

As *k* values at low light intensities depend on the total pigment content of the algae whereas *k* values on the "plateau" are more a function of chlorophyll *a* or the nitrogen content of the cell one cannot expect any one simple mathematical relationship for *k* as a function of light intensity as has sometimes been assumed. This is obvious from a consideration of Fig. 6–10.

It will be seen from Fig. 6, 7 and 10 that the C-14 uptake rates for *Skeletonema* and *Dunaliella* are the same as those for the production of particulate carbon over short periods, the discrepancies in *Skeletonema* results at 0.075 ly/min being explicable because of the low "unstable" 3-hr C-14 data for this species. This confirms the observation made in a large natural culture by Antia *et al.* (1963). The determinations of the cellular carbon increase for *Monochrysis lutheri* was spoilt. Results are not given for *Amphidinium carteri* because of the presence of appreciable quantities of non-cellular organic material in the culture, excreted by the cells as a gelatinous matrix. For this reason the *k* values in Fig. 9 are probably too low.

The C-14 method generally gave very poor agreement with both net and gross photosynthesis as measured by the oxygen technique, contrary to many opinions held in the literature (Ryther, 1956; Nielsen and Al Kohly, 1956). The differences cannot all be attributable to calibration errors as they depended on light intensities and, as mentioned earlier, it is most improbable that they can be attributed to the PQ values ascribed to oxygen data. These would have had to be abnormally high and vary in a complex manner with light intensity. The latter is unlikely in view of the constancy of "plateau" values and the good relationships found between oxygen data and pigments.

Agreement between C-14 uptake and net photosynthesis depended on species, nutrition and light intensity, being always worse at low light intensities (Fogg, 1958). It was good with *Dunaliella* at high light intensities, except with low phosphorus in the medium, but was poor with the *Dunaliella* culture conditioned at high light intensities. The agreement at high light intensities was also passable with ***Amphidinium***, less satisfactory with ***Monochrysis*** and extremely bad with *Skeletonema* at all intensities, especially when there was a shortage of phosphorus. It is to be regretted that the relatively few culture experiments that have been undertaken to compare techniques in the past (cf. Thomas, 1963) have used well-illuminated Chlorophyceae, a set of circumstances which in this work gives the best agreement between the methods.

It would seem reasonable to assume that a light and nutrient dependent excretion of labelled organic matter is taking place when C-14 results are lower than net oxygen rates but are in agreement with particulate carbon formation. The involvement of phosphate is suspected because agreement between C-14 uptake and net photosynthesis is at its worst when there is a low concentration of phosphate in the media but not much different with and without excess nitrate (Thomas, 1963).

The discrepancy between rates measured as net photosynthesis and C-14 uptake also depends on the light source. At the time of each main experiment determinations of net photosynthesis and C-14 uptake were also made with bottles illuminated for 3 hr by cool-white fluorescent lamps. The ratios of C-14 rates and net photosynthesis in these experiments were always appreciably higher (5–15%) than ratios found in the main experiment. The reason for this behaviour is maybe related to the spectral distribution of the two light sources. It will be seen from fig. 2 and 5 of Jitts *et al.* (1964) that cool-white fluorescent light activates appreciably more of the long-wave chlorophyl *a* section of action spectra than does the filtered tungsten light used in the present incubator.

It is interesting to compare ***k*** values for photosynthesis and cell division. The latter were comparatively low and independent of light intensity above about 0.07 ly/min, as would be expected from the investigations described in the previous paper.

Gross photosynthesis appears to take place by a mechanism largely independent of cell division (Sorokin, 1960). In the *Skeletonema* experiments the increase of particulate carbon and the C-14 uptake rates both coincided with cell division rates but were only about 60% of net photosynthesis (cf. results by Antia *et al.*, 1963, for a natural culture, mainly of diatoms). Although a direct check for dissolved organic carbon in the media was not made it is difficult to see how this discrepancy can be explained other than by the excretion of organic matter, as has been reported by several workers using Chlorophyceae (Allen, 1956; Tolbert and Zill, 1956; Fogg, personal communication).

C-14 results could also be low if the counting efficiency varied with different algae, even with nominally "weightless" sources. Such a complication would make the C-14 method, as normally used, almost impracticable. One of the causes of the low rates found with *Skeletonema* may arise from abnormally low counting efficiencies, if preliminary results from the combustion and gas-counting of filters are to be believed. The matter will be the subject of thorough study later. However, the C-14 results in the present work are those which would be obtained by the techniques normally employed by laboratories working on primary productivity and are therefore valid in the present context of methods intercomparison.

The production of carbon by *Dunaliella tertiolecta* after 6 hr at high light intensities (Fig. 6) was much higher than cell division, being equal to net photosynthesis, and no excretion of labelled material took place (Fig. 4). At lower light intensities particulate carbon formation proceeded at a rate only slightly greater than cell division and appreciably lower than net photosynthesis with, presumably, some excretion. The state of affairs at high light intensity, namely the rate of particulate carbon formation greatly exceeding the rate of cell division, cannot continue indefinitely without gigantism. It is significant in the *Dunaliella* experiment that at 10 hr the *mean* rate of particulate carbon formation is beginning to decrease towards the cell division values, which would result in a stable cell size.

In order to examine this condition more thoroughly a whole 10-litre culture was preconditioned in the incubator for 24 hr at about 0.15 ly/min before an experiment was carried out in the usual manner. Results are shown in Fig. 7. The k values for gross and net photosynthesis at high light intensities were *unchanged*, being the same (0.18 hr^{-1}) as with the culture reared at lower light intensity. However the light intensity for the onset of the plateau was much greater (0.25 ly/min) and the rate of gross photosynthesis per unit of chlorophyll *a* was different (Table II).

Dunaliella cells reared at low light intensities thus appear to have an inherently greater k value for gross photosynthesis at moderate light intensities than do the cells grown in brighter light (Fig. 6 and 7 at about 0.05–0.1 ly/min). This is presumably the same effect demonstrated with *Dunaliella* by Yentsch and Reichert (1963). These authors found that cells kept dark for 24 hr had a greatly increased capacity for gross photosynthesis (k values) at 800 ft-c. This behaviour, as mentioned by Yentsch and Reichert, provides a reasonable explanation for much of the diurnal periodicity of photosynthetic capacity reported in nature by many workers.

At 0.075 ly/min formation of particulate carbon may have been somewhat slower than cell division and the rates of the two processes were much closer together at high light intensities than with the culture grown at low light intensity. From the C-14 rate curve it would seem as if particulate carbon formation coincided with cell division at light intensities up to about 0.2 ly/min and both were markedly less than net photosynthesis, in contrast with the behaviour illustrated by Fig. 6.

It is difficult to see, *a priori*, any reason for the results just described. Several authors have distinguished between the "sun" and "shade" states of phytoplankton cells (Sargent, 1940; Ryther and Menzel, 1959; Nielsen and Hansen, 1959b; Ichimura *et al.*, 1962) and the subject has been given a thorough treatment by Nielsen *et al.* (1962) using *Chlorella*. "Sun" cells have less chlorophyll than "shade" cells but give approximately the same plateau k values so that the factor B is greatest for a cell in its "sun" condition. However, the terms "sun" and "shade" are relative and depend on the light intensity at which cultures have been preconditioned (Sorokin, 1958). By comparing the rates found during preculturing with rates in the incubator experiments it appears as if the cool-white fluorescent light used in the present work gave an *effective* preconditioning intensity of about 0.075 ly/min with respect to incubator light, although the energy rating of the fluorescent light was undoubtedly less than this (presumably a spectral effect). This standard preconditioning gave "shade" cells when they were exposed to light intensities of 0.35 ly/min. All cultures exposed to this intensity showed a reduced rate of chlorophyll *a* formation relative to cell production (see Fig. 6, 8 and 10). The chlorophyll per cell thus decreased, although there was no direct "bleaching" (Nielsen and Jörgensen, 1962; Sistrom, 1962). At about 0.075 ly/min, on the other hand, the cell division and chlorophyll formation kept pace with each other. At 0.02 ly/min the cells behaved as "sun" cells and chlorophyll *a* increased more rapidly than cell division (Fig. 6), being formed at the same rate as gross photosynthesis. The culture of *Dunaliella* preconditioned at 0.15 ly/min (Fig. 7) was no longer a "shade" cell at 0.35 ly/min and it behaved as a "sun" cell at 0.075 ly/min where the rate of chlorophyll *a* formation again equalled that of gross photosynthesis and was much more rapid than cell division. Although the "excretion" of organic carbon at high light implied in Fig. 7 as compared with Fig. 6 clearly depends on preconditioning it cannot be attributed to a property of "sun" cells *per se* as worse excretion may have occurred with *Skeletonema* when in its "shade" form. The effect presumably depends mainly on species and perhaps on the external concentration of metabolite such as phosphate (Keaney and Tolbert, 1962).

The algae studied here had widely different amounts of pigment per cell and widely differing ratios of the various pigments one to the other. In all cases, however, chlorophyll *b* and chlorophyll *c* changed in proportion to chlorophyll *a* when the amount of the latter varied in a species due to alterations of the light intensities or the state of nutrition of the algae.

The ratios of carotenoid to chlorophyll *a*, on the other hand, varied considerably, being particularly sensitive to changes of cellular nitrogen (Table I) as previously noted by many workers. *k* values for carotenoids, not plotted, were similar to or sometimes slightly greater than cell division *k* values at all light intensities and did not show the "sun" or "shade" trends found with chlorophyll *a*. It would seem as if the kinetics of carotenoid production relative to cell division are almost unaffected by conditions of light or nutrition and the variability of chlorophyll carotenoid ratios depends mainly on the kinetics of chlorophyll formation.

Dunaliella cells were low in carbon when phosphorus deficient or when reared in high light intensities (Table I) but in neither case were they notably small. Nutrient deficient cells were more spherical and somewhat less motile than cells grown in the presence of excess nitrogen and phosphorus. *Skeletonema* had more carbon per cell when grown to a state of phosphorus deficiency and the morphological changes were very striking. Chain formation ceased and cells became very large and showed all the symptoms of silicate deficiency (Braarud, 1949). It is clear that silicate metabolism is intimately connected with phosphorus metabolism (Jörgensen, 1952; Lewin, 1955).

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Temperature, Salinity and Plankton in the Eastern North Atlantic and Coastal Waters of Britain, 1957. IV. The Species' Relationship to the Water Body; Its Role in Distribution and in Selecting and Using Indicator Species[1]

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ABSTRACT

Temperature and salinity have been used to characterize the surface water bodies of the oceanic eastern North Atlantic and coastal waters of Britain. Species of plankton have been entered on temperature-salinity diagrams and it has been demonstrated that each is related in a characteristic manner to one or more of the water bodies, or their extended influences in mixtures. This relationship is maintained by each species regardless of seasonal temperature fluctuations of the water bodies, and within the available salinity variations. Species may be present concurrently in oceanic water of one temperature and oceanic-coastal water of a different, lower temperature. Increases and decreases in numbers of a species occur within the limits of these water bodies. These factors are regarded as demonstrating, for the area investigated, that species' occurrences and distributions may not be dependent on temperature and salinity.

Discontinuities occur in the distribution of species, chiefly at boundary zones between different water bodies. These arise because some species may cross the boundary zone into another water, while other species of similar origin do not (or cannot).

An hypothesis is proposed to explain these features. Unspecified properties are postulated for those water bodies which are contributing to mixed waters in the area studied. The relationships of the species are regarded as being regulated by their tolerances towards the properties; and occurrences and distributions of the species are discussed relative to the presence of the properties. It is considered that the species' reactions to the properties provide a reasonable basis on which to explain the relationships to the waters in the area, their apparent independence of seasonal temperature changes, and the species' distribution.

INTRODUCTION

The temperature-salinity-plankton (T-S-P) technique demonstrates one sort of relationship between the organisms and their environment. When applied over a complete seasonal cycle this relationship will be shown for an environment in which the measured properties (temperature and salinity) are fluctuating, or subject to other variations. Since the technique deals with field data, the reactions demonstrated for organisms can be expected to be "normal" ones in the conditions prevailing.

Earlier papers of this series (Bary, 1963a, b, c) appear to be the first application of T-S-P diagrams to the study of occurrences of zooplankton in relation to water bodies over a prolonged period, in this instance one year.

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The course of fluctuations in abundance of species, and stability of the species' reactions under the stress of a varying environment have been observed; so also have their reactions to environmental changes resulting when water bodies mix. Such features have implications for, or effects on, the interpretations of the species' distribution and use as indicator species. Of more importance, they raise questions of the role played by temperature and salinity in regulating occurrences of organisms in the area concerned and whether other environmental factors may not be more effective in determining the relationships demonstrated in the T-S-P diagrams.

It is the purpose of this paper to examine the effects on species' occurrences of the seasonal fluctuations of temperature and salinity. As a result of this examination it becomes necessary to develop an hypothesis which bypasses temperature and salinity and proposes instead that tolerances of species to particular, but unspecified, properties are the basis of the mechanism regulating their relationships to the waters in the area surveyed. The distribution of the species is reviewed with this hypothesis in mind.

TEMPERATURE, SALINITY AND SPECIES' OCCURRENCES

Species' Relationships and Temperature

The examples of T-S-P diagrams (Fig. 1–3) show that the relationships of the species of each of the planktonic groups to the water bodies were similar whether the temperatures were at their coldest in late winter or warmest in late summer. This was most apparent for the warm (southern) group (Fig. 1). In March the species of this group occurred between about 11.5 and 12.7°C and in August, between about 16.5 and 18°C, but not at temperatures below 16.5°C. That is, the species remained associated with southern water, despite the change of temperature. The warm-transitional species, e.g. *Pleuromamma* spp. (Fig. 2), similarly maintained their relationships, but in this instance with southern and transitional waters (and for *P. robusta*, with northern water). The relationships of the warm-transitional-neritic and general-neritic groups were broad, as exemplified by *Metridia lucens* (Fig. 3), but again they were maintained throughout the course of the seasonal fluctuations. The coastal (neritic) group also showed consistent relationships during wide temperature fluctuations in the inshore waters.

The "waters of additional properties", in the northwest, and south of the area surveyed (Bary, 1963a), were inadequately sampled biologically during 1957 and species' occurrences relative to these waters cannot be assessed.

Interspecific differences in the relationships of species to the water bodies were maintained throughout the year. In the T-S-P diagram for January, *P. gracilis* occurred up to 10°C in transitional water, but *P. robusta* was present over the available temperature range of 9–11°C, (in February (Fig. 2) *P. gracilis* barely entered transitional water). In September (Fig. 2) the temperature of transitional water was between 12 and 16°C; again *P. robusta* was present over the whole range, but *P. gracilis* only up to 13.5°C (in diluted) and 14°C (in undiluted) oceanic water. In oceanic waters, *M. lucens* (Fig. 3) was more plentiful in southern and warm transitional water, *A. clausi* in cooler transitional and northern waters. However, *M. lucens* extended into northern water and *A. clausi*, when abundant, into warmer transitional water. Even so, both species remained closely associated with the appropriate waters, regardless of the temperatures of the waters. The maintenance of the relationships was a feature common to all species. It is reflected in the species' distributions (Bary 1963c).

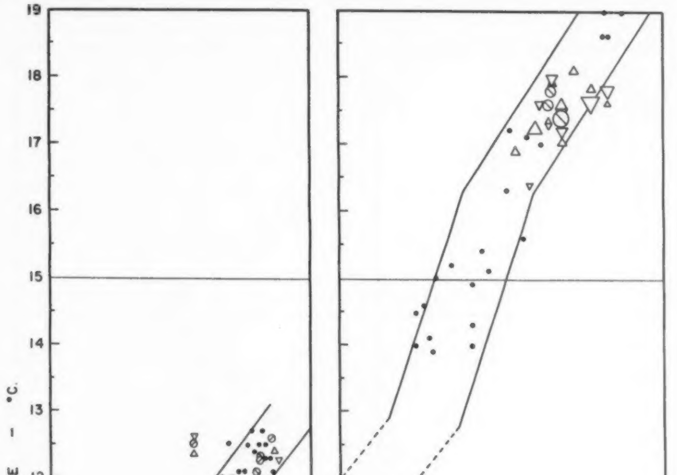


FIG. 1. T-S-P diagrams of the winter-summer relationships of the warm (southern) group of species. Note the presence of specimens in a pocket of relatively low-salinity water in March and in a pocket of water of slightly greater salinity (than southern water) in August.

A decrease in temperature within a water body can be assumed when proceeding towards higher latitudes. In the geographic distributions (Bary, 1963c) there is a tendency for several of the oceanic species to occur in higher numbers towards the southern parts of the area, in the warmer waters. The majority of species discussed have southern centres of distribution and initial increases in numbers occurred there which probably accounts for such a tendency. Otherwise, and as a whole, the groups were distributed throughout the area occupied

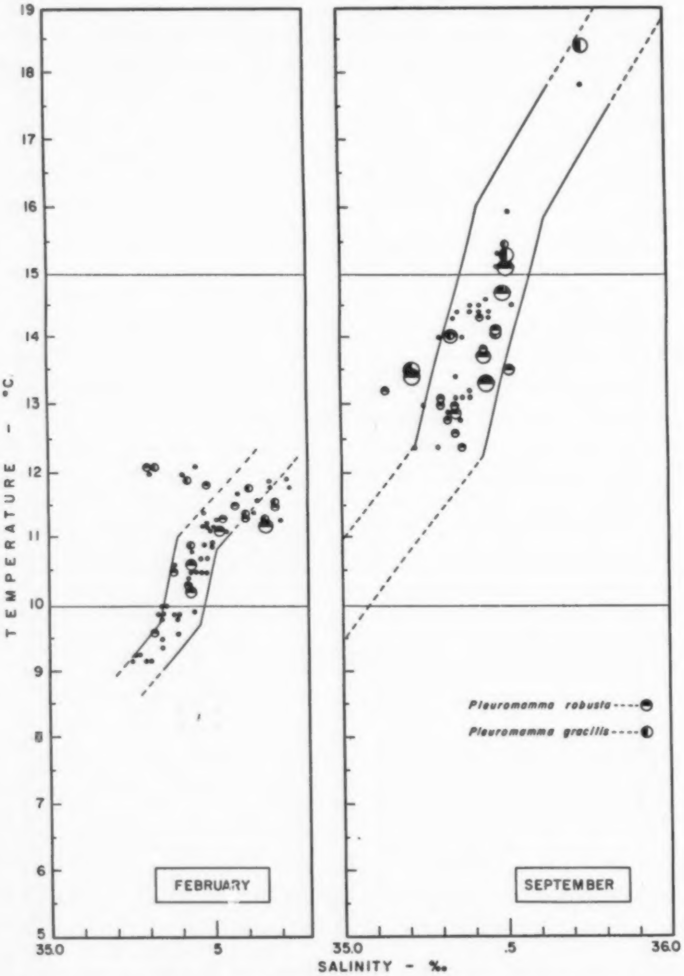


FIG. 2. T-S-P diagrams of winter-summer relationships of two warm-transitional species. Note the presence of specimens in relatively low- and high-salinity pockets.

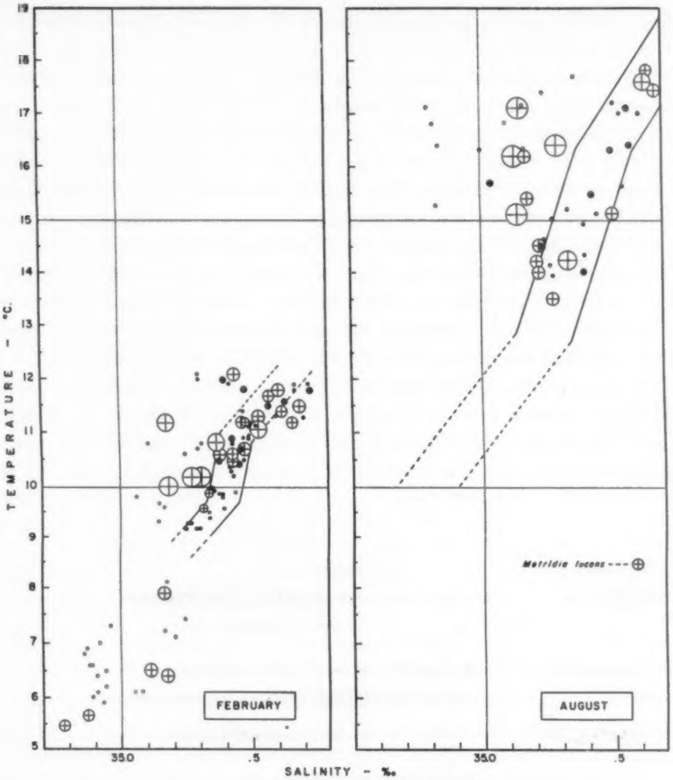


FIG. 3. T-S-P diagram of winter-summer relationships of a representative of the "oceanic-neritic" species. Note occurrences at very low temperatures in February in coastal (North Sea) water.

by the environmental conditions appropriate to them. Latitudinal temperature changes within the area occupied by a water body did not obviously affect species' distributions.

Not infrequently species of the warm-transitional-neritic and general-neritic groups occurred contemporaneously at temperatures in coastal water different from those in oceanic water (Fig. 3). *Spiratella retroversa* (Bary, 1963b) was not present in transitional water (at 9–11°C) in January or February, but was collected in the North Sea at approximately 6°C; in March it was present between 10 and 11°C in oceanic water, but at 7.5°C in the North Sea. *Metridia lucens* (Fig. 3), *A. clausi* and *Centropages typicus* occurred similarly.

FLUCTUATIONS IN NUMBERS AND TEMPERATURE

A qualitative estimate of relative abundance in several species is illustrated diagramatically in Fig. 4. The blocking for each species (upper half of Fig. 4) can be read in relation to mean annual temperature fluctuations of the waters.

Data for the warm (southern) group of species are spasmodic, but clearly there was an increase in numbers from February to March when the southern water was coldest and another about August when the water was warmest. *Pleuromamma* spp. increased noticeably at the low temperatures of February and March, but were reduced to a few specimens in April and May, even though temperatures were rising. A second increase on rising temperatures occurred during June, July and August, but highest numbers were collected from September to October on falling temperatures. *Sagitta serratodentata* (not shown in Fig. 4) was collected in low numbers in March and in relatively high numbers in July, during rising temperatures, but was most common from September to November when temperatures were falling. *Metridia lucens* increased first during February–March, followed by a second increase commencing about October at temperatures considerably higher than during February or March. There were three peaks of high numbers for *Spiratella retroversa* each at a different part of the temperature cycle. The chief increase of *Centropages typicus* occurred in July and August at maximal temperatures and continued into October, during falling temperatures. For *Acartia clausi* there was a single, long period of high

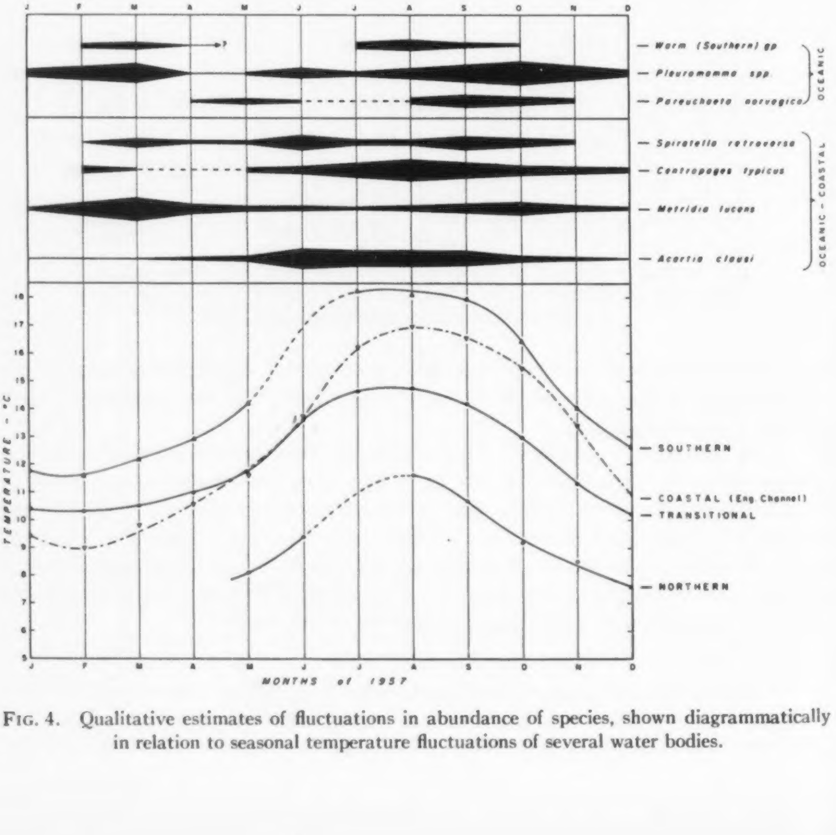


FIG. 4. Qualitative estimates of fluctuations in abundance of species, shown diagrammatically in relation to seasonal temperature fluctuations of several water bodies.

numbers, between July and September, which began and ended at roughly similar temperatures.

These examples illustrate two general features. Firstly, the species' cycles of abundance pursued a variety of courses. Secondly, cycles of low and high abundance of the species appeared to have little connection with the seasonal temperature fluctuations.

Associated with species' fluctuations is the disappearance of species, and specimens, during winter months (Fig. 4). In oceanic waters in 1957, the reduction in numbers commenced about November and was more or less complete by December. Probably a similar reduction from 1956 had continued into January 1957. The species' disappearance in November–December could have been due to downward migration, perhaps stimulated by falling temperatures. In contrast the same species "reappeared" in February at lower temperatures than when they disappeared, and when (on mean values) it was slightly colder than in January, in which month, generally speaking, the species were absent. Factors other than temperature seem to be necessary to account for a species' return to surface waters at a temperature lower than that at which it disappeared in the autumn. If different stages in the life cycle were concerned in autumn and spring, then different temperature requirements may have been effective. The reactions of the species appear to be readily explained as not due to temperature, but other, temperature-dependent factors may be involved, particularly with respect to the different life-history stages not recorded during analyses.

In the North Sea, English Channel and Western Channel the increase in specimens commenced in March and April. *Temora longicornis* occurred over much of the temperature range, and *Sagitta* spp. above about 10°C. The mean temperature in April of these waters was between only 0.5 and 1.5 C° higher than during February, but in the North Sea it was between 4 and 5 C° lower than in the Western Channel, with an intermediate value in the English Channel. Again, temperature does not seem to have been important to the onset of the increase. Temperature may seem to be affecting occurrences of *Sagitta* spp., but this probably results from lack of material from the North Sea during the greater part of the year.

The temperature range approximated 3.5 C° in winter and 9 or 10 C° in summer for the combined oceanic water bodies, and 13 to 14 C° in coastal and oceanic-coastal waters in winter. It could be argued that temperature ranges beyond the species' tolerances were not encountered. This may be true. It must be noted, however, that during summer months the oceanic species either could have remained in water of similar temperature to that found during winter, or could have dispersed into water of such temperature. They did not do so, and instead remained in association with their appropriate environmental conditions. Further, in winter, there was a wide range of temperatures available in the coastal areas and neritic species could have been suitably accommodated in some part of this range, but again, they largely disappeared. These points suggest the temperature range had little effect on the species' relationships to the water bodies.

The T-S-P diagrams demonstrate the species maintained relatively constant relationships to the water bodies in the area during the temperature fluctuations of 1957. In some months, particularly in winter, species were present at temperatures in oceanic waters which differed from concurrent temperatures in oceanic-coastal or coastal waters; fluctuations in numbers of species and specimens appear to have proceeded without being affected by seasonal temperature changes; the reduction of species during November 1957 commenced at temperatures which were higher than when the species had appeared in the previous February; in oceanic waters numbers of specimens increased in February at slightly lower (mean) temperatures than in January when few specimens were present, but in coastal waters, species appeared together at different temperatures in the three areas.

The evidence above, from field data, suggests that although species may be limited in the range of their occurrences, the limitation probably was not due to temperature effects. Each species maintained its particular relationship to the water bodies throughout the year, and as a corollary, differences in the relationships between species were also maintained. An important feature is that at the summer temperatures of an oceanic water body, the species did not occur at lower temperatures equivalent to those which obtained for the water body in winter. This was a general feature, but was best shown among species exhibiting close relationships to restricted ranges of conditions.

SPECIES' RELATIONSHIPS AND SALINITY

The species of the warm (southern) group were closely associated with southern water, but in February and March (Fig. 1) some occurred in diluted oceanic water and in February in a high-salinity pocket. The extreme range over which specimens were present was in the vicinity of 0.33 ‰. The warm-transitional species (Fig. 2) also occurred in clouds of low-salinity water (January, February, March, September, October) and high-salinity water (February, April). The extreme range was 0.4 ‰. All, or most, species of the groups occurred as well throughout the salinity ranges of the water bodies with which they were associated. Similarly, the cold (northern)- transitional species (*Pareuchaeta norvegica*) was present over the range for northern water, and much of the range for transitional water (in September).

There appears to be a rânge of about ±0.1 ‰ around 35 ‰ over which species typical of coastal and oceanic-coastal waters intermingle among themselves and with species from oceanic water (Fig. 3, 6). Species of the two oceanic-neritic groups extended into waters in coastal areas; numbers were often high, but a noticeable and usually abrupt decrease occurred at salinities near, and less than 35‰, coincident with a changeover in occurrences between the coastal species *Sagitta elegans* and *Sagitta setosa*. At the shoreward limit of unmixed oceanic water, roughly coincident with the edge of the shelf, elimination of species endemic to oceanic water took place (Fig. 5). At this limit, consistent and sometimes relatively large, decreases in salinity were apparent (Bary, 1963a).

While the salinity range presented to the oceanic species was not large, the possibility seems to have been available for preferences to be exhibited,

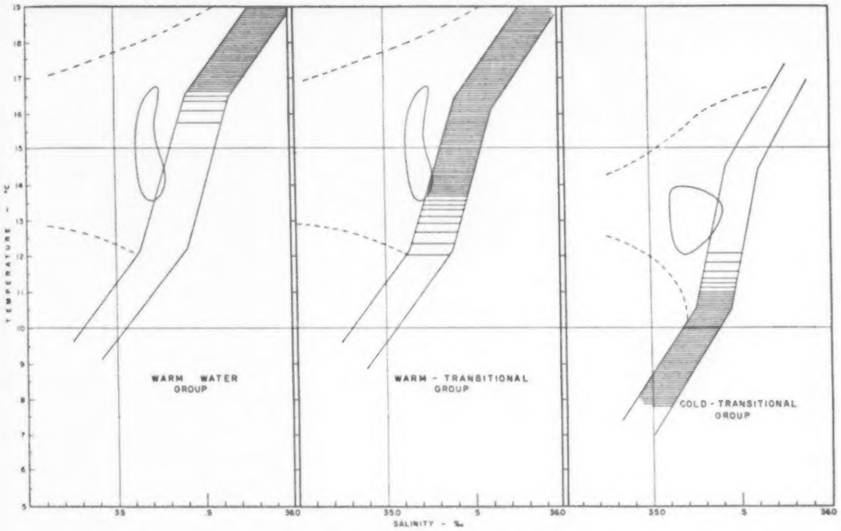


FIG. 5. Diagrammatic representation of species' relationships in oceanic waters.

particularly with respect to the low and high salinity pockets. This differentiation did not appear to occur which suggests that salinity was having little effect on the occurrences of the species. The discontinuities in species' occurrences above the shelf edge and at about 35 ‰ in coastal waters suggest that perhaps a relationship exists between their occurrences and the salinity. It is believed this would be a superficial explanation and an alternative is offered in the next Section.

SPECIES' RELATIONSHIPS AND HYPOTHETICAL PROPERTIES

If the T-S diagram provides a proper evaluation of the surface water bodies, and if the field occurrences of species are related in some manner to the water bodies, then this relationship should be apparent in the T-S-P diagrams. Such relationships between the species and the water bodies are considered to have been demonstrated (Bary, 1963b) and Fig. 5 and 6 present them diagrammatically for the 6 groups analysed. However, the evidence in the previous section suggests that, for the area under study, neither temperature nor salinity appeared to have detectable effects on the species' relationships to the water bodies, and consequently on their occurrences. This may seem a paradoxical statement to ensue from a technique in which temperature, salinity, and occurrences of species, are correlated. It is believed, however, that the diverse features discussed under Temperature, and Salinity and Species' Occurrences can be explained otherwise than due to the effects of these two properties.

Hypothetical Properties of Primary Water Bodies

In order to formulate an explanation, it is proposed that the primary water bodies (northern, southern and coastal), as characterised in T-S diagrams, each

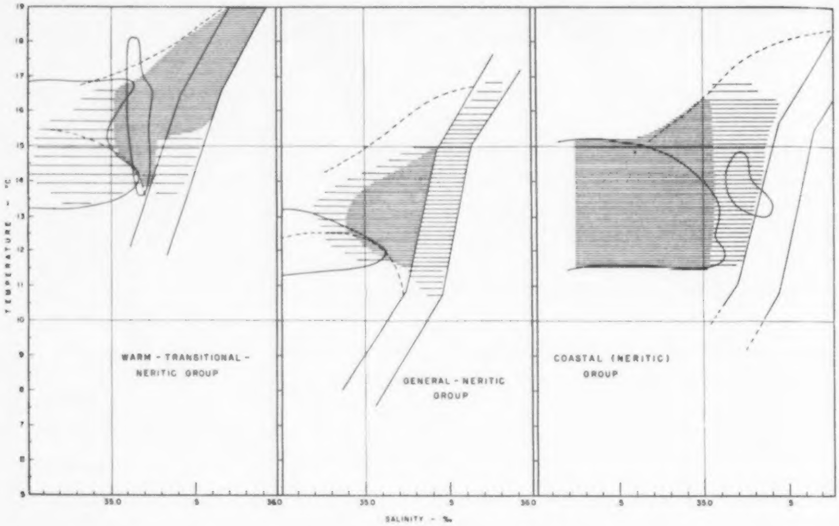


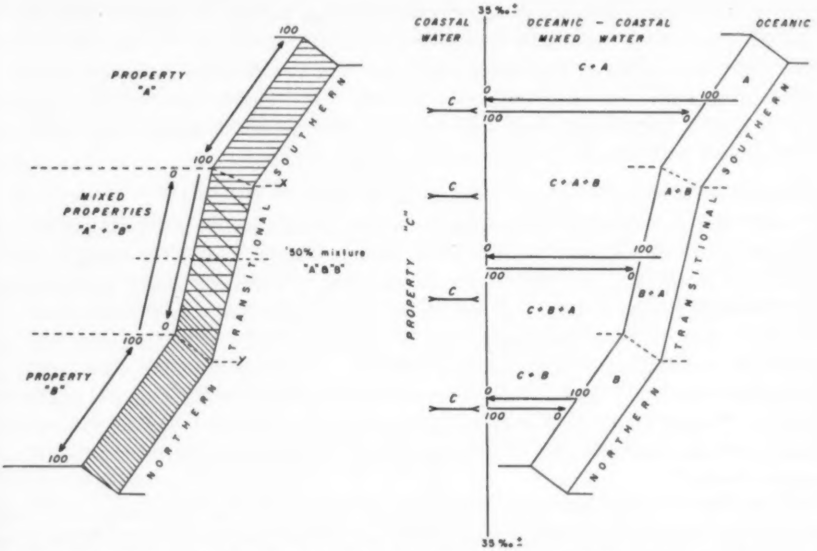
FIG. 6. Diagrammatic representation of species' relationships in oceanic, oceanic-coastal and coastal waters.

include a particular property (or aggregate of properties) which is relatively constant to that water body and in some sense unique to it. It is further proposed that it is to such properties that each of the species reacts in an individual manner, depending on its tolerance towards the properties. These properties are able to provide a single mechanism on which an explanation may be based of the variety in the species' relationships to the water bodies and the apparent absence of effects from temperature and salinity. Insofar as the basis of the relationships may by this means be understood, so the occurrences and hence the distributions of the species may also be understood.

The proposed unique property (or group of properties) of southern water will be designated *A*, and of northern water, *B* (Fig. 7a). Throughout southern water and up to position *X*, in the T-S diagram *A* would be at full strength; between positions *X* and *Y*, as mixing proceeds, *A* would become progressively diluted until at *Y* it is nil. Similarly *B*, of northern water, would become progressively diluted from full strength at *Y* to nil at *X*. Coastal water is considered to include property *C*; the dilution of this property by oceanic water is discussed below.

OCEANIC SPECIES AND THE HYPOTHETICAL PROPERTIES

A species which originated in southern water would react to property *A*. It would seem likely that if it occurred in the presence of this property in a mixture of two oceanic waters such as in transitional water, it should also occur in a mixture of southern and coastal waters. This, in fact, does not always follow. If on the other hand the species exhibited tolerance towards property *A* and was


PROPERTY "A"
100
100
0
X
MIXED PROPERTIES "A" + "B"
50% mixture "A" & "B"
0
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SOUTHERN
TRANSITIONAL
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35 ‰ ±
COASTAL WATER
OCEANIC - COASTAL MIXED WATER
OCEANIC
C + A
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FIG. 7. *Left:* Diagram showing the hypothetical unique properties of the primary northern and southern water bodies mixing into the transitional water; *Right:* diagram showing mixing of unique properties between oceanic and coastal water via the oceanic-coastal water.

intolerant of *B* or *C*, then its absence from water including these properties would be understandable. Should another species of similar origin exhibit tolerance towards property *B*, then it would be able to occur in a mixture of waters containing *A* and *B*, to the extent permitted by its tolerance towards *B*. Or if the species were tolerant of *A* and *B* and of *C*, it would occur in a mixture containing all three.

If the warm (southern) species (Fig. 5) were tolerant of property *A* of southern water, but intolerant of properties *B* and *C*, they would be collected from southern water, but not, other than exceptionally, from waters containing northern or coastal waters. Species of the warm-transitional group (Fig. 5) would be tolerant of diluted *A* and of some concentration of *B* in transitional water, but intolerant of *C* of coastal water. *Pleuromamma robusta* (Fig. 2) of this group could tolerate full strength of property *B* in northern water; but *P. gracilis* would appear to tolerate only about 50% strength of property *B*. *Pareuchaeta norvegica* (cold-transitional species—Fig. 5) would be tolerant of property *B* of northern water, but of low tolerance to the higher concentrations of *A* in transitional water. *Metridia lucens* (Fig. 3) and other species of the oceanic-neritic groups (Fig. 6) would be tolerant not only of properties *A* and *B* in oceanic waters, but also to some extent of *C* in coastal water.

The oceanic occurrences of *Acartia clausi* (an oceanic-neritic species) suggest it inhabited cooler transitional water. This would require it to be tolerant of mixed properties *A* and *B*. As it was rarely collected in southern water and was sparse in warmer transitional water, except during periods of peak abundance, it probably has a low tolerance to higher concentrations of *A*. It occurred, however, in northern water which suggests tolerance towards undiluted property *B*, although possibly for relatively brief periods. The higher numbers of *A. clausi* in the cooler transitional than elsewhere in oceanic water would agree with its tolerance of property *B*.

OCEANIC-NERITIC SPECIES AND THE HYPOTHETICAL PROPERTIES

In mixing between oceanic and coastal water bodies three hypothetical properties, *A* of southern water, *B* of northern water and *C* of coastal water would be involved (Fig. 7b). In a simple case, if southern water were mixing with coastal water, property *A* would become progressively diluted towards nil as the mixing became attenuated; property *C* would be at full strength in indigenous coastal water and become diluted as southern water was reached. Where northern and coastal waters were in contact, properties *B* and *C* would be mixed. Transitional water, containing both properties *A* and *B*, would introduce them into the coastal water in concentrations according as northern or southern water dominated.

The T-S-P diagrams (Fig. 1 to 3, 5, 6) illustrate that the oceanic species comprising the warm (southern), warm-transitional and northern (cold)-transitional groups rarely crossed from oceanic into oceanic-coastal or coastal waters. These species would seem to be tolerant towards some concentration of properties *A* and/or *B* in the oceanic water bodies, but when water containing the properties mixes with coastal water (property *C*), they are excluded. This suggests intolerance of property *C*. The almost entirely oceanic occurrences of species such as *Sagitta serratodentata* and *Pleuromamma* spp. exemplify the situation.

Species of the warm-transitional-neritic group (Fig. 3, 6) appeared to disperse from southern water into the transitional and oceanic-coastal, mixed waters. The extent to which these species occurred in the mixed waters suggests that they were not only tolerant of property *A*, but also of some concentration of properties *B* and *C*. The widespread occurrences of *Centropages typicus* and *Spiratella retroversa* in oceanic-coastal waters indicate high tolerance towards *C*; *Metridia lucens* and *Acartia clausi* were probably even more tolerant towards it since they penetrated further into coastal water. *Euchaeta hebes* and *Candacia armata* were associated in general with oceanic-coastal water in which less coastal water was mixed, indicating lower tolerance to *C* than for *Metridia* or *Spiratella*.

The close association of the coastal (neritic) species to waters in the coastal areas illustrates the reverse of the oceanic species' lack of tolerance to inshore waters (Fig. 6). The coastal species rarely occurred in oceanic waters which indicates intolerance towards full concentrations of properties *A* and *B*. *Sagitta setosa* would appear to be tolerant of little more than the most dilute of these properties whereas *S. elegans* would appear to have equally low tolerance of near full-strength oceanic or coastal properties.

The abrupt reduction in numbers of specimens of several oceanic-coastal species (Fig. 3, 6; and Bary, 1963a, b), and the changeover in occurrences of *Sagitta elegans* and *S. setosa* at approximately 35 ‰ salinity, probably results from the relative tolerances of the species towards coastal property *C* and oceanic properties *A* and *B*. It is interesting that the discontinuity in distribution apparently should be consistent at that salinity; it may be a manifestation of tolerance limits of several species to particular concentrations of the unique properties. It suggests that some fairly well defined faunal boundary zone exists.

General Features of Species' Relationships and the Hypothetical Properties

The proposed unique properties *A*, *B* and *C*, in the eastern North Atlantic area, appear to offer a single mechanism determining relationships between species and environmental conditions. To be acceptable, the properties would have to regulate occurrences in particular water bodies (or their extended influence) at all times, regardless of fluctuations in temperature, and over the ranges of salinities encountered. The T-S-P diagrams have demonstrated that, in fact, the relationships were relatively constant. The "tolerance" through which the species' reactions to the unique properties appears to be expressed, and hence the relationships determined, probably must be regarded as physiological in origin, and unique to each species.

It is not meant to imply that temperature and salinity can have no effect on species or their occurrences, but within the ranges obtaining in the water bodies, they apparently did not produce observable effects. Extreme high or low temperatures or salinities within a water body could probably become limiting factors and determine occurrences of species. Or rate of change, especially of temperature, could modify reactions. The temperature fluctuations discussed have been gradual ones, and the salinity variations were not excessive, but species presented with steep gradients of either property could well react in ways different from those demonstrated. Moore (1955), for example, discussed such possibilities in relation to the depth to which a vertically migrating species may descend.

From the above discussions, it would appear that a species would need to react, either positively or negatively, to all unique properties being contributed to a mixture of waters. The intensity of a species' reaction, and the extent to which it may penetrate into the mixture, would then depend on a condition of equilibrium due to the relative tolerance of the species towards concentrations of properties from the other contributing waters. These observations can be summed up in the conclusion that, with only occasional exceptions, a species will be found (in the area studied) in those environmental conditions which are suitable, namely the water body with which it has been associated.

The nature of the hypothetical properties is not known. Investigations on larval development by Wilson (1951) and Wilson and Armstrong (1952, 1954) undoubtedly show that some sort of factor or factors differentiates between the mixed oceanic-coastal ("*elegans*") water, in which pluteus larvae grew normally, and coastal ("*setosa*") water, in which growth was abnormal. Lucas (1955) has discussed "external metabolites" and effects the presence of these may have on species. The "properties" which lead to the experimental results of Wilson (1951), and Wilson and Armstrong (1952, 1954), would seem to be basic constituents of, and apparently relatively permanent to, the water bodies. They would agree better with the "properties" discussed herein than external metabolites; the latter would appear to be temporary and changing properties, appearing in the water as a result of immediate biological activity and acting perhaps as "short-term modifiers", determining, for example, the seasonal succession of species within the water body.

Given appropriate circumstances it appears the unique properties of a water body may be modified, perhaps fairly rapidly. Northern and southern waters are regarded as originating in the recent past in a single water mass (Bary, 1963a), but on the whole organisms from southern water did not enter northern water. Conversely, occurrences of *Pareuchaeta norvegica* were centred in this water. These are indications that northern water had developed a degree of uniqueness in its properties (named property *B* above). The ability of species such as *Pleuromamma robusta*, *Metridia lucens* and *Acartia clausi* to enter northern water, however, could be interpreted as indicating it had not reached "maturity" as a water body. The means by which such modification may occur are not known.

FAUNAL FILTER ZONES

It would seem reasonable to suppose that if some elements of the planktonic fauna of one water body can cross the boundary into a second water body, there should be no "barrier" in a physical sense to prevent the remainder from doing so. However conditions in these zones apparently are such that a "sorting" or "filtering" action operates on the fauna whereby some but not all species are able to cross. It is proposed that, with respect to the fauna, these zones be termed "faunal filter zones".

Several faunal filter zones have become evident. The warm (southern) group of species normally were not collected outside the area occupied by southern water, whereas other species dispersed from southern water across the boundary zone into transitional water; and species such as *Pleuromamma robusta* and *Metridia lucens* were collected beyond the zone between transitional and northern waters. Wholly oceanic species rarely crossed the zone between oceanic and oceanic-coastal water, but species of the oceanic-neritic groups crossed freely. Lastly, a faunal filter zone, perhaps essentially similar to those above, may be present between oceanic-coastal and indigenous coastal water.

Of these several faunal filter zones, the one between oceanic and oceanic-coastal waters appears to have a major distributional discontinuity associated with it (Colebrook *et al.*, 1961; Glover, 1961). The zone delimits the shoreward occurrences of species endemic to oceanic water and the seaward extent of neritic species; oceanic-neritic species cross the zone.

Boden (1952) and Boden and Kampa (1953) have suggested that the type of circulation over and at the edge of the shelf conserved the insular plankton of Bermuda. Possibly a similar circulatory phenomenon is operative in Europe; such would account for discontinuities in properties, especially salinity (Bary, 1963a), and could be invoked to help explain the distributions of the species.

Because only some species are transferred across the zone suggests, however, that other factors of a non-physical nature are involved. An oceanic species (e.g. *Centropages typicus*) which is tolerant towards the unique property *C* in coastal water will be able to penetrate the boundary zone between oceanic and coastal waters; a species (e.g. *Pleuromamma robusta*) intolerant of the property is presented with alternative possibilities. In the first place it does not penetrate the zone because it cannot, or if it does, it survives only briefly. Or secondly, the species immediately reacts against intolerable conditions and rapidly disappears; it would seem likely the species may actively avoid the conditions, perhaps by a migration downwards. The latter might be assisted if a suitable circulatory system were associated with the zone. A similar complex of factors would seem to exist in all boundary zones.

From the above it is seen that the reactions of species to the hypothetical properties provides an explanation of the selective action on the species of the faunal filter zones. Indeed it is difficult to see in what other way the zones might operate since major changes of temperature, and often of salinity, usually were not associated with them during 1957.

FLUCTUATIONS IN ABUNDANCE AND WATER BODIES

The causes of fluctuations in the species' abundance could not be determined. During analysis of collections, life-history stages usually were not recorded. There are two consequences in the present study. Firstly, although an increase in numbers might be due to breeding, this could not be stated definitely; secondly, areas in which a species may have been breeding could not be located. Therefore, only increases and decreases in numbers of specimens are available for comment (Fig. 4).

Increase in abundance of a species may have resulted from breeding alone, or a combination of breeding plus augmenting of the surface population from deeper levels, by an upward migration for the purpose of breeding. Combined activity of this sort could better account for the rapid increases of *Metridia lucens*, *Pleuromamma* spp. and *Acartia clausi* (Fig. 4) for example, especially as they coincided with a spread of the species over broad areas during relatively short periods. Conceivably such combined activity would be a more likely explanation of this than dispersal of a species from a geographic centre. Likewise, these species disappeared rapidly, which would ensue if they migrated downward, subsequent to a period of breeding activity near the surface.

With the species' occurrences dependent on their reactions to the hypothetical properties, then the location of breeding, and perhaps whether breeding takes place, would also be dependent on the properties, rather than on the temperature or salinity of the water body. The tolerances of species towards the unique properties would ensure they returned to the surface into those conditions which were suitable, and this again would result in breeding taking place relative to particular water bodies.

Vertical migrations, other than diurnal, have been documented previously by, for example, Nicholls (1933), Sømme (1934) and Marshall and Orr (1960), for various life-history stages of *Calanus finmarchicus* and *C. hyperboreus*; and

Pareuchaeta norvegica undergoes seasonal vertical migrations (Wiborg, 1955). These migrations have led to distributional complexity. It seems reasonable to expect that species such as *Metridia lucens* or *Pleuromamma* spp. could also make periodic vertical movements and to add to the complexity of their distributional picture. If so, the movements would appear to have been more frequent than "seasonal" in that several cycles of increases and decreases (Fig. 4) were apparent in some species.

OCCURRENCES, DISTRIBUTIONS AND INDICATOR SPECIES

The sum of a species' occurrences during an interval of time, when viewed geographically, may be regarded as equivalent to its distribution. If, as proposed, its occurrences are regulated by the presence of certain properties in the water body, it follows that the distribution of the species is also regulated by these properties.

But, by definition, plankton consists of organisms which are distributed, by and large, by water movements. If surface currents were the only feature to be considered in the distribution of organisms in the area surveyed, it seems unreasonable to find that one species from a particular water body is able to enter an admixture of this with a second water, while another species, presumably with equal opportunities to enter, does not do so. This feature was mainly evident at the boundary zones and its effect is to cause discontinuities in distribution.

Pleuromamma gracilis illustrates a particular situation. This species extended into the mixed transitional water to about a "50% mixture" of the northern and southern waters. The "boundary zones" for *P. gracilis* would need to be broadened to include the whole of the mixed transitional water and not just the "zone of immediate mixing" about its periphery which is responsible for several other examples of discontinuity of distribution. The disappearance of *P. gracilis* from transitional water resembles the dropping out of species associated with north–south distributions (John, 1936). Although this is only one example, it raises the query as to whether there is an equivalent dropping-out of species within the primary water bodies, such as northern and southern waters, or whether dropping out may, be more properly considered a feature of mixtures of such primary water bodies.

Insofar as the several points above refer to distribution, they also refer to the particular aspect of distribution in which species are used to indicate the presence in a locality of conditions with which they have been associated at another place. These are the Indicator Species.

INDICATOR SPECIES

A variety of species has been suggested as indicators of 'foreign' waters and of water movements for the European area (Hjort and Gran, 1900; Gough, 1905; Meek, 1928; Ostenfeld, 1931; Russell, 1935, 1939; Corbin, 1947; Fraser, 1952, and others). The successful establishment of a species as an indicator has been by empirically associating its occurrences with the oceanographic conditions in which it has occurred. This requires prolonged observation, even where there is a thorough knowledge of the fauna, not only of the area being studied, but of contiguous areas.

Temperature and salinity may be referred to in discussing the indicator species (Fraser, 1952), but usually their occurrences are thought of in terms of the waters entering the area concerned; for example in the North Sea they are associated with "Atlantic water". Even so, conclusions can almost only be of a general nature (Fraser, 1952).

Direct relationships of species to waters have been shown by correlating occurrences with temperature and salinity either for oceanic water masses (Haffner, 1952; David, 1955; Hida, 1957; McGowan, 1959), or for surface waters (Bary, 1959, 1963a, b and c) by means of the T-S-P technique. Species may be selected on the basis of the relationships demonstrated to indicate fairly precisely a limited type of environment, or the progressive nature of changes during mixing of one water with another. Two points are important. Firstly, any species may be used as an indicator provided the environmental conditions with which it is associated are known; secondly, any such indicator species will be subject, in more or less degree depending on its tolerances, to the sort of controls on its occurrences which have been discussed.

The selection of indicator species with the aid of the T-S-P diagram can be made from material collected over a *brief* period. Since only those species actually present at the time can be used, the selection is independent of fluctuations in abundance. In a continuing, or long-term programme, fluctuations in abundance must be taken into account since a species may become so depleted that even though the appropriate water is present, it is not being reliably indicated. It is necessary to know when cycles of abundance occur in a species, the water body or bodies in which they occurred, and if possible their cause. For example, low numbers of a species in an area may be the result of a periodic decrease in its numbers, or to a low tolerance towards waters present in the area. High numbers might result from an influx of specimens from elsewhere, or from the species being tolerant to the prevailing conditions and perhaps breeding in the locality. Such contingencies may be met by selecting a series of indicator species, for example with differing cycles of abundance, but similar relationships to the waters in the area.

The relationships of species to the waters have been shown to remain relatively constant throughout the year. Therefore, species selected as indicators with help of the T-S-P diagrams will be effective for the appropriate conditions whenever or wherever they occur, irrespective of seasonal temperature fluctuations or salinity changes (excepting that strong gradients in, or extremes of, either property could act as limiting factors). The differing tolerances of the species may be turned to advantage. Thus, highly tolerant oceanic species could indicate conditions from oceanic water to an attenuated mixture with another water; or, a species intolerant of conditions in the mixed water would delimit the area occupied by the unmixed oceanic water. There are many intermediate stages, some applying to mixtures of two oceanic waters, others to mixtures of oceanic and coastal waters.

The very tolerant species may disperse from a centre in one water body, but may be widespread and in high numbers in other waters (e.g. *Metridia lucens*). The presence at times of such species may indicate correctly that their 'parent' water has recently entered an area and mixed with the resident water. However, the tolerant species may maintain itself or increase in numbers by breeding in the mixed water, or it may enter upper layers periodically from deeper levels. In such circumstances it may not be possible to detect a fresh influx of water plus the species. The species therefore probably cannot be regarded as reliably indicating an influence that has been, or is in process of being renewed and should be treated with suitable caution as an indicator. Information about the tolerances, cycles of fluctuations in abundance and the waters in which these occurred, would increase the reliability of estimates of conditions made with the aid of the very tolerant species.

DISCUSSION AND SUMMARY

In the eastern North Atlantic and coastal waters of Britain three primary water bodies have been delineated and for each it is proposed there is a unique property — property *A* in southern water, property *B* in northern water, and in coastal water, property *C*. Transitional water, considered a mixture of northern and southern waters, would contain *A* and *B*, and oceanic-coastal water a mixture of oceanic properties *A* and/or *B* with coastal property *C*.

The nature of the unique property is unknown. It must be a relatively permanent feature of a water body, but it appears it can be modified, perhaps fairly rapidly.

Relationships of species to water bodies, shown by the T-S-P diagrams, can be explained in terms of the species' reactions to the presence of the unique properties, but not by the effects of temperature or salinity on the species. If it is assumed that interspecific tolerances towards the properties differ, the fact that some species are restricted to one water body whilst other species may move into another water (which may be a mixture) can be readily explained. So too can the filtering action of the boundary zones between water bodies.

Several other investigators have shown close relationships between a variety of species and water masses of the Southern Ocean, in the Pacific, Indian and Atlantic oceans. There is sufficient material available in these investigations to suggest that even on a scale broadened to include the oceanic water masses, relationships to the masses may result from the reactions of species to undetermined properties, rather than to effects of temperature or salinity.

Since in the eastern North Atlantic area, the relationships to the water bodies determined in which one the species occurred, and since the water bodies have been shown as geographic entities, then the relationships controlled the distributions of the species. Within this framework, species no doubt are transported by water movements, but the reactions of the species at boundary zones, towards the unique properties of the opposed water, appear to modify the distributions which might have been expected from water movements alone.

Species that disappear at boundary zones possibly do not survive the transfer into intolerable conditions in the opposing water, or they may actively avoid these conditions by migration away from them. Fluctuations in abundance of species may also modify the distributional picture. Possibly the fluctuations are contributed to by upward and downward vertical migration, which may be breeding migrations. These same movements would help account for rapid increases and decreases of species over extensive areas.

In these circumstances the geographic location of a plankton sample becomes relatively unimportant. What is important are the distributions of a water body, of known range of properties, and of the species that are demonstrated as being associated with it. Such considerations apply especially to those species selected with the aid of the T-S-P diagram for the particular purpose of being used as indicator species.

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NOTES

Observations on Herring Spawning off Southwest Nova Scotia

Although large quantities of pre-spawning, spawning and spent herring are caught commercially in the waters off southwest Nova Scotia, little is known of the size of the stocks, the distribution of the spawning grounds, the density of eggs or the number of larvae produced. This account deals with a herring spawning bed survey which was carried out in the Trinity Ledge district off Black Point, southwest Nova Scotia, on September 5 and 6, 1961. The only other such survey in the western North Atlantic was conducted in the Chaleur Bay area of the Gulf of St. Lawrence in May 1962 and reported by Tibbo *et al.* (1963).

To survey the area, a grid of stations was laid out at 0.8-km intervals between Black Point and Trinity Ledges. Van Veen and Petersen dredges as well as a lined scallop drag were used for sampling and a spawning bed was located 5.6 km south of Cape St. Mary and 2.3 km W × S Black Point, approximately lat. 44°01′N, long. 66°12′W.

The exact location where eggs were first found in this area of strong tide was marked by dropping a buoy on the spawning bed and lines of dredging stations were run out from the buoy in all directions beyond the points where no more herring eggs were found. The dredges cut out sections (Fig. 1A and B) of the sheet of eggs that was on the bottom and the thickness of the sheet was determined at each station.

Figure 2 shows the areas of eggs of approximately equal thickness enclosed by "contour" lines. The bed was roughly "oyster-shaped" with the egg sheet being thickest (about 3.25 cm) near the small end and tapering off in all directions — more gradually to the northwest than in the other directions.

The total area of the spawning bed (about 67,500 sq m) was determined by means of a planimeter. The total number of eggs on the bed (2.1×10^{11}) was calculated from samples that were preserved and examined later in the laboratory.

Fecundity determinations have been made on very few Canadian Atlantic herring. Because of this the mid point in the range found by Baxter (1959) for autumn-spawning herring in northern Europe was used. In that area females of 30.7 cm (average length of 300 fish obtained from the Trinity Ledge area) have about 92,000 eggs.

Assuming a sex ratio of 1:1 (50.5% males, 49.5% females in the sample of 300) the school which spawned on the Black Point spawning bed consisted of about 4.6 million individuals weighing about 13,700 metric tons.

Tibbo *et al.* (1963) found the herring in Chaleur Bay to be spawning in May in water 1–6 m deep. This area had a dense covering of *Phyllophora*, *Laminaria*, *Chondrus*, *Fucus* and *Ascophyllum* over a bottom of gravel, small stones and large masses of red sandstone. Surface temperatures ranged from 5.4 to 6.3°C with a tidal current of 0.5 knot. Winter flounders (*Pseudopleuronectes americanus*

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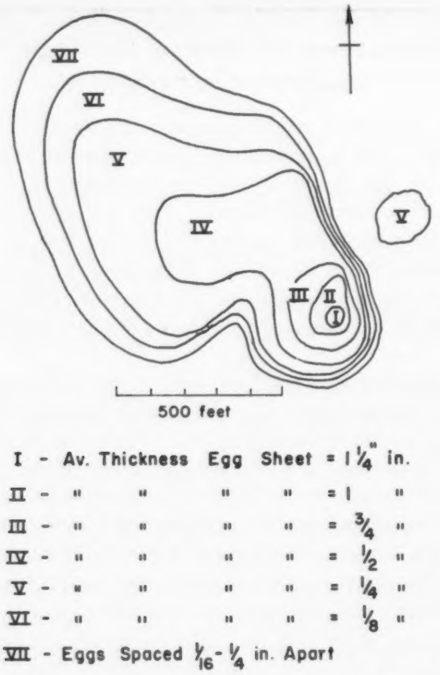

FIG. 2. Herring spawning ground off Black Point, N.S.

(Walbaum) 1792) were quite numerous in the area and were the chief predator on the eggs.

In contrast the spawning bed off Black Point was located in September, in water 11–13 m deep. The bottom was fairly flat covered with sand and a few small stones, and was completely devoid of any vegetation whatsoever. It should be noted, however, that an unsurveyed spawning bed nearby was covered with a dense growth of *Desmarestia aculeata* (L.) which was completely permeated with herring eggs (Fig. 1C). The surface temperature on the surveyed bed ranged from 12.0 to 12.8°C, the bottom at 14 m from 11.3 to 12.0°C and the tidal current was from 1.5 to 2.0 knots. Haddock in the area had large quantities of herring eggs in their stomachs and seemed to be the main predator.

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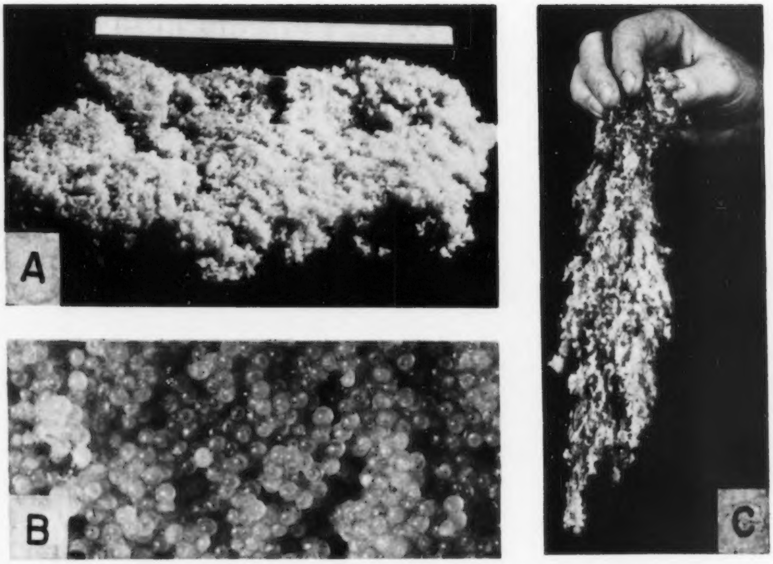

FIG. 1. Herring eggs from spawning beds 1 and 2 off Black Point, N.S. (A) Egg sheet from bed 1; (B) Enlarged section of A; and (C) Frond of *Desmarestia* from bed 2 covered with eggs.

McKenzie—J. Fish. Res. Bd. Canada

First Record of *Mytilicola orientalis* Mori in the California Mussel *Mytilus californianus* Conrad[1,2]

The presence of parasitic copepods of the genus *Mytilicola* in the gut of molluscs has been known for many years. One species, *M. intestinalis* Steuer, is known to cause extensive mortalities in commercial mussel beds in Europe (Korringa, 1950). The species found in Washington is *M. orientalis* Mori (Mori, 1935) and was originally described from Pacific oysters (*Crassostrea gigas* (Thunberg)) and a mussel (*Mytilus crassitesta* Lischke) in Japan. Odlaug (1946) noted the presence of this copepod in oysters (*C. gigas* and *Ostrea lurida*), bay mussels (*Mytilus edulis*), clams (*Venerupis staminea*) and eastern cups (*Crepidula fornicata*) in Washington. Recently, Sparks (1962) was able to describe for the first time pathological changes in the gut of *C. gigas* associated with the presence of *M. orientalis*.

While sampling native oysters (*O. lurida*) on February 19, 1963, at the California Fish and Game Reserve No. 1 in Humboldt Bay, California, it was noticed that bay and California mussels (*M. edulis* and *M. californianus*) were attached to nearby rocks, affording an excellent opportunity to check the possible infestation of *M. orientalis* in all three species of bivalves. California mussels had not previously been reported to harbor the copepod, though large numbers of sectioned California mussels were examined by the authors. Samples of native oysters, bay and California mussels were preserved in a solution of 9 parts 95% isopropyl alcohol and 1 part glacial acetic acid for subsequent gross examination. The results are presented in Table I.

TABLE I. Percent infection, average number of *Mytilicola orientalis* per infected bivalve, and average size of *M. orientalis* in *Ostrea lurida*, *Mytilus edulis*, and *Mytilus californianus* collected February 19, 1963, from California Fish and Game Reserve No. 1 at Humboldt Bay, California.

Species	Shell length	No. examined	Percent infection	Av. No. *Mytilicola* per infected bivalve	Av. size of *Mytilicola*
	mm				*mm*
Ostrea lurida (Native oyster)	42–52	52	9.6	2.4	6.3
Mytilus edulis (Bay mussels)	30–47	24	58.3	2.3	4.6
M. californianus (California mussel)	45–55	20	65.0	2.5	4.3

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Several of these shellfish were also fixed in Zenker's fluid, blocked in paraffin, sectioned, stained in Harris Hematoxylin and Eosin and examined for the presence of the copepod. The results were as follows:

	Positive	*Negative*	*Total*
Native oysters	1	11	12
Bay mussels	2	4	6
California mussels	4	2	6

Both the gross and microscopic examination revealed the presence of *M. orientalis* in California mussels, demonstrating rather clearly that the California mussel is at least equally susceptible to infection as the bay mussel and possibly more susceptible than native oysters.

Figure 1 demonstrates that, although relatively few copepods were found, two distinct size classes were easily identified. Examination of these copepods revealed that most of the copepods between 3.0–4.0 mm in length were males

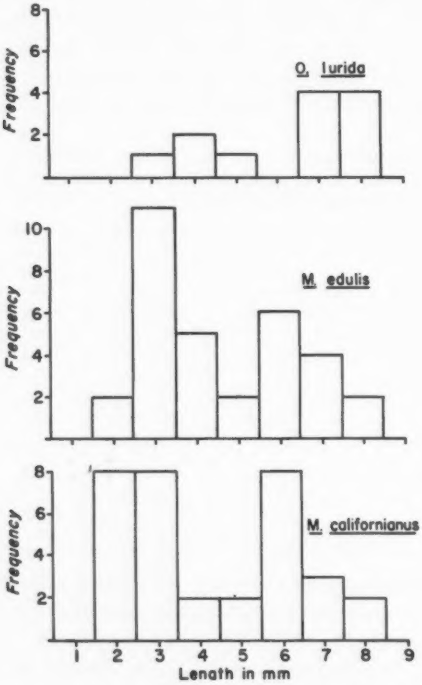

FIG. 1. Length frequency of *Mytilicola orientalis* from *Ostrea lurida*, *Mytilus edulis*, and *Mytilus californianus* collected Feb. 19, 1963, from reserve No. 1 at Humboldt Bay, California.

and those of 6.0 mm and longer were females. It is possible that during the gross examination of shellfish, many of those less than 3.0 mm in length were overlooked. This assumption is based on the fact that Caspers (1939) and Cole and Savage (1951) were able to find *M. intestinalis* in bay mussels as small as 0.3 mm. However, it is also possible that the early copepodid stages were not present during this time of the year because of the breeding cycle of the copepod.

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Nessorhamphus ingolfianus Schmidt, An Oceanic Eel, Recorded in Canadian Continental Waters

During the course of redfish (*Sebastes mentella* Travin) stock surveys with the research vessel *A.T. Cameron* in October and November 1962, Mr E. J. Sandeman obtained 4 specimens of the duck-billed oceanic eel, *Nessorhamphus ingolfianus* Schmidt. These appear to be the first records from Canadian continental waters despite the fact that *Nessorhamphus* larvae were found in large numbers over a wide area of the North Atlantic during the Danish larval eel investigations and the fact that the type specimen was taken only about 130 miles southeast of Flemish Cap (Schmidt, 1931). These fish are chiefly inhabitants of warm sub-tropical oceanic waters and according to Schmidt (1931) the main spawning area is in the Sargasso Sea. Beebe (1935) reports on their capture in the Bermuda area, and discusses synonymy, developmental stages, osteology and ecology. Beebe also considers that the *Avocettina scapularostris* described by Borodin (1929) from Gulf Stream waters south of the Grand Bank was this species.

Nessorhamphus is distinguished from oceanic eels in other families by the following features: its characteristic flattened snout, which is longer than the lower jaw and which bears the olfactory capsule at its expanded tip; the possession of well-developed pectoral fins situated at a distance behind the rear of the skull; the small ventro-lateral gill pore; the position of origin of the dorsal fin close behind the pectorals; the position of the vent which lies far behind the gill opening; and the truncate compound caudal formed by the union of the dorsal and anal fins. Many of these characteristics are apparent in the diagrams of the head of the largest of our specimens (Fig. 1), and other characteristics can be deduced from the measurements given in Table I.

The specimens reported here were caught at the positions given in Table I in an otter trawl fitted with a $1\frac{1}{8}$-inch mesh liner in the codend. It may only be coincidence that both tows in which specimens were caught were at depths of about 200 fathoms (366 m), and they may have been caught in mid water. For the same reason, it is difficult to be sure of the temperatures at which they were living, particularly because of the sharp but meandering temperature boundaries which lie along the southwest edge of the Grand Bank and the Nova Scotian Shelf where the specimens were caught. During the course of a 30-min tow the net might pass through several meanders, pockets, or tongues of the warm slope-water gyre, but the temperature data come from single bathythermograph casts made at the ends of the tows.

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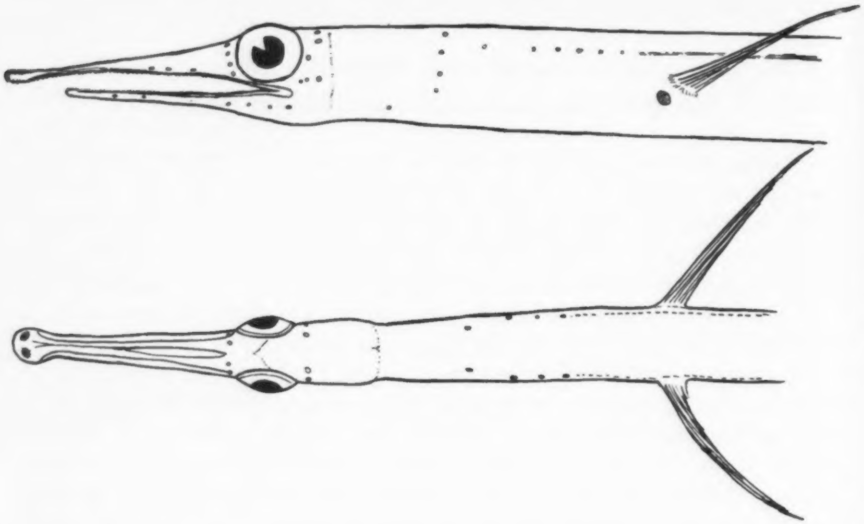

FIG. 1. Head of *Nessorhamphus ingolfianus* (length 291 mm) from the Nova Scotian Shelf, viewed from the side and above.

TABLE I. Capture details and body proportions of 4 specimens of *Nessorhamphus ingolfianus* caught by the *A.T. Cameron* in October–November 1962.

Date:	Oct. 18, 1962		Nov. 6, 1962	
Position:	43° 41′ N		43° 13′ N	
	52° 21′ W		60° 58′ W	
Depth fished (*fath*):	205–215		193–204	
Bottom temperature (°*C*):	2.6		5.6	
Specimen:	A	B	C	D
	mm	*mm*	*mm*	*mm*
Total length	167	171	212	291
Predorsal length	33	39	46	60
Preanal length	95	95	121	164
Prepectoral length	23.0	26.5	30.8	40.8
Snout length	9.5	11.5	12.0	15.8
Horizontal eye diameter	2.8	2.7	3.5	4.4
Upper jaw exceeds lower	2.2	3.0	3.6	3.8
Head depth	4.3	5.0	5.6	5.6
Body depth	6.5	7.5	8.0	11.5
Length of pectorals	5.2	6.2	8.1	11.0

The presence of this species in the Canadian area is probably associated with the warm eddy of slope water which is derived principally from the northern edge of the Gulf Stream and as such the records are not too surprising. It would seem unlikely that specimen A was caught in the 2.6°C water at the bottom. The associated bathythermograph trace shows water temperatures of 5°C between 82 and 110 fathoms (150–200 m) and another trace made a short distance seawards shows water temperatures of 6°C at 120 fathoms (220 m), while the surface temperature was 12°C. At the position where the other 3 somewhat larger specimens were caught the bottom temperature was 5.6°C, but a large mass of water, with temperatures exceeding 8°C and reaching as high as 10°C, was found at 55–120 fathoms (100–220 m), and the surface temperatures were also high (10°C).

The 2 larger specimens, C and D, are extensively black-pigmented, but the smaller specimens have little pigment except on the head, and are little beyond the glass-eel stage. The effects of relative growth are conspicuous particularly with respect to head size and in the larger specimens the heads appear to be disproportionately small.

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